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Allergy and asthma



Cover illustration

Scanning electron micrograph of a house dust mite superimposed on a photomicrograph of an asthma sufferer's airway, which is blocked with fibrin and eosinophils. (Images courtesy of A. Syred/SPL and A. J. Frew.)

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Most of us are familiar with the consequences of allergic disease. We all know people who are affected by a condition such as hay fever, asthma or eczema. The costs to public health and the economy are massive and growing, and a major research effort has been underway for many years to understand these diseases. Despite much progress, allergic diseases still present significant puzzles, which hinder the development of effective therapies. This supplement provides a framework for tackling these puzzles — an overview of current thinking, from the ultimate causes of allergic disease to the plethora of approaches that are being contemplated for therapeutic intervention.

Allergies are disorders of the immune system. In healthy individuals, the immune system is poised between maximizing protection from unwelcome invasion or internal imbalance, and minimizing harmful over-reaction to external or internal events. But in allergic disease, this delicate balance is disturbed, with consequent adverse effects on morbidity and mortality.

Although allergic diseases can affect people in different ways, they have many features in common. Underlying these diseases, there seems to be a sequence of events that may begin as early as during fetal life, and that leads inexorably to allergy. Understanding these early events will provide new opportunities to curb the development of allergic disease.

The development of allergic disease depends on many factors, both genetic and environmental. Pollution clearly plays its part, but the cast of players and their mechanisms of interaction are highly complex. It is the great number of the factors influencing allergy that makes it difficult to decide where to focus efforts aimed at cure and prevention.

Whether from the perspective of a researcher, a health-care provider, or indeed a sufferer, we are confident that you will find much useful information within these pages. We are very pleased to acknowledge Schering-Plough, whose financial support helped to make this supplement possible.

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The epidemic of allergy and asthma

Stephen T. Holgate



The foundations of *allergy** have evolved slowly, beginning with John Bostock's description of *catarrhus aestivus* or hay fever (1819), Charles Blackley's recognition of pollen grains as causative agents (1873), the discovery of a transferable tissue-sensitizing factor in the serum by Prausnitz and Küstner (1921) and, finally, the identification of this factor as an immunoglobulin subclass (*immunoglobulin E* or IgE) by Johansen in Sweden and the Ishizakas in the United States (1967)¹. The past 30 years has witnessed a spectacular increase in our knowledge of the cellular and molecular mechanisms of allergic disease (Fig. 1), which has been paralleled by the rising trends in the incidence and health impacts of these diseases worldwide². Whereas in Bostock's and Blackley's day hay fever was a rare disorder restricted to the privileged class, as we approach the millennium almost half the population of the West demonstrates sensitization to one or more environmental *allergens*. In countries such as Britain or Australia, this translates to 1 in 4 children under the age of 14 years having *asthma* and 1 in 5 having *eczema*³. Added to this is the occurrence of serious allergic

Allergic diseases, such as asthma, rhinitis, eczema and food allergies, are reaching epidemic proportions in both the developed and developing world. Key factors driving these rising trends are increased exposure to sensitizing allergens and reduced stimulation of the immune system during critical periods of development. In allergic disease, there is a polarization of T-lymphocyte responses, and enhanced secretion of cytokines involved in regulation of immunoglobulin E, mast cells, basophils and eosinophils, ultimately leading to inflammation and disease. A clear understanding of the cellular and molecular mechanisms of allergic disease and the complex interplay between genetic and environmental factors will undoubtedly create new opportunities for public health and therapeutic interventions.

disorders caused by new allergens such as nuts, soya and latex.

At one extreme, *anaphylaxis* and asthma can be life threatening and every year there occur deaths often in young people that are avoidable. Fortunately, most allergic disorders are not life threatening, but they all cause distress and misery for millions — often at a time in their lives when they should be most active. *Allergic rhinitis*, asthma and eczema all interfere with sleep, intellectual functioning and recreational activities, whereas food allergy leads to considerable anxieties for fear of inadvertently ingesting the offending allergen.

The basis of allergy

With the exception of insect anaphylaxis, the process of sensitization usually results from contact of allergens with mucosal surfaces. In the case of respiratory allergy, it is exposure to aeroallergens that is important, asthma being associated more with indoor allergens derived from dust mites, pets and fungi, whereas seasonal rhinoconjunctivitis requires exposure to grass and tree pollens. The nature of the allergen itself seems important, with many inhalant allergens exhibiting enzymic activities (for example, *Der P₁* and *Der P₉* from dust mites⁴). In an experimental setting, simultaneous exposure to allergen and proteolytic activity enhances sensitization, possibly by disrupting epithelial tight junctions. This might in turn allow greater access of allergen to *dendritic cells* or interrupt critical molecules involved in IgE regulation such as the low-affinity receptor for IgE (CD23) and the interleukin (IL)-2 receptor (CD25)^{4,5}. In the case of food allergens, the biological challenge for the allergens is to survive digestion.

Communication between allergens and T

cells occurs through *antigen-processing* cells, such as dendritic cells. A major breakthrough in our understanding of the pathophysiology of allergy, irrespective of the organ in which the phenotype is expressed, has been the discovery that, in response to allergens, T lymphocytes produce a restricted array of *cytokines* encoded in a small cluster on the long arm of chromosome 5 at bands 31–33 (chromosome 5q31–33), which has been shown to be the IL-4 gene cluster. The proinflammatory cytokines are produced in particular by a subtype of T helper cells known as *Th2*. The other T helper subtype, *Th1*, tends to antagonize the allergic response (Fig. 1). Factors that select for Th2 responses include low-affinity binding of the allergen peptide to the groove of major histocompatibility complex (MHC) class II, selective use of co-stimulatory molecules (for example, engagement of T-cell CD28 by CD86 in preference to CD80) and reduced dendritic cell secretion of IL-12. The microenvironment in which the dendritic cell finds itself is also emerging as an important determinant of the balance between interferon- γ (IFN- γ)-producing Th1 cells and proallergic Th2 cells. One suggestion is that prostaglandin E₂ produced by epithelial cells, inhibits IL-12 production by dendritic cells, thereby favouring a Th2 response. The property of dendritic cells to acquire a phenotype that is capable of directing T-cell cytokine polarization has led to the suggestion that there are two types of dendritic cell designated DC₁ and DC₂ (ref. 6). But in mice, lung tissue *macrophages* with some properties of dendritic cells are capable of shutting down local T-cell responses, a mechanism that may help explain why all individuals testing positive for an allergic reaction are not asthmatic⁷.

*Terms in italic are defined in the glossary on p. B39.

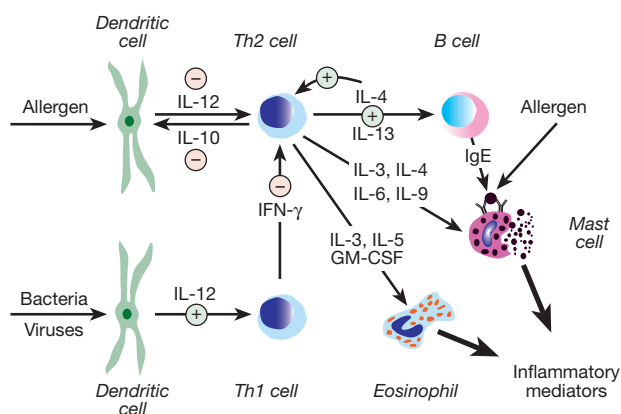


Figure 1 Proposed cellular and molecular mechanisms of allergy. Both soluble mediator and cognate interactions between dendritic cells and naive CD4⁺ T cells direct their differentiation to the Th2 phenotype with the capacity for enhanced secretion of cytokines encoded in the IL-4 gene cluster on chromosome 5q31–33. Th2 polarization is effectively suppressed if T cells are driven along the Th1 pathway by IL-12 to produce increased IFN- γ . It is suggested that exposure to bacterial and possibly viral ‘danger’ signals at a critical time during immune development in childhood enhances Th1 at the expense of Th2 polarization producing protection against allergy.

Helper T cells are not the only source of proallergic cytokines. *Mast cells, basophils, eosinophils*, CD8⁺ T cells and, under certain circumstances, bronchial epithelial cells, fibroblasts and smooth muscle can all produce cytokines encoded by the IL-4 gene cluster⁸.

IgE as the allergic trigger

Both asthma and eczema are considered to be primarily T-cell-mediated disorders with IgE serving as an important trigger. In asthma, this view is supported by the efficacy of a humanized blocking antibody directed against T cells⁹. Detailed knowledge of the site of molecular interaction between IgE and the high-affinity IgE receptor (Fc ϵ R1) has also enabled mouse monoclonal antibodies to be developed that selectively bind to the relevant IgE epitopes. By splicing sequences encoding the antigen-binding regions of one of these monoclonal antibodies into a human IgG framework, a therapeutic antibody (MAb E-25) has been developed¹⁰. Administration of MAb E-25 to allergic asthmatic subjects led to rapid elimination of circulating IgE to almost undetectable levels and was paralleled by a marked reduction in Fc ϵ R1 expression. Over a period of 9 weeks, this treatment markedly attenuated the early-phase (mast-cell dependent) and late-phase (inflammatory) bronchoconstriction to inhaled allergen. The efficacy of MAb E-25 in treating chronic asthma and rhinitis is evidence for a key role of IgE in chronic allergic inflammation, although not all patients responded equally well⁹.

The effectors of allergic disease

Although IgE production is associated closely with allergic hypersensitivity responses, a wide range of cellular responses underlies

chronic allergic disease, including the production of inflammatory mediators. Release of histamine and cysteinyl leukotrienes account for most of the early- and late-phase responses, although the cellular origin of these mediators may differ — mast cells in the early phase and eosinophils, basophils and macrophages in the late phase.

The discovery of histamine by Dale and Laidlaw in 1911 created a new science — immunopharmacology. Histamine H1-antagonists are first-line drugs for the treatment of anaphylaxis, rhinoconjunctivitis and urticaria but are singularly ineffective in asthma and eczema. The discovery of slow reacting substance by Feldberg and Kellaway in 1938 and its subsequent chemical characterization in 1979 by Bengt Samuelsson's group as a mixture of three cysteinyl leukotrienes — LTC₄, LTD₄ and LTE₄ — has stimulated the development of antagonists for this class of lipid mediator¹¹. It seems that this new asthma treatment is especially effective in a sub-population of asthmatics who are intolerant of aspirin and other non-steroidal anti-inflammatory drugs and who exhibit enhanced LTC₄ synthase activity^{11,12}. Understanding the factors that regulate the synthesis and release of these mediators from mast cells, eosinophils, macrophages and monocytes, as well as understanding genetic variants of the cysteinyl-LT₁ receptor and its promoter, will create an opportunity for using genetic screening as a method for predicting drug responsiveness (pharmacogenetics).

Human mast cells can be classified on the basis of their granule content of neutral proteases. All mast cells contain the unique four-chained neutral protease tryptase, but only those located in connective tissue are enriched with chymase and carboxypepti-

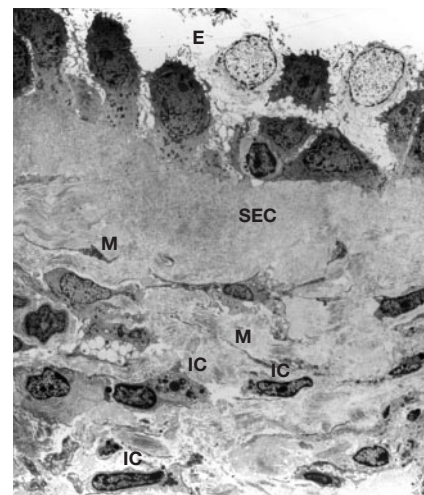


Figure 2 Transmission electron micrograph of allergic asthmatic bronchial mucosa showing epithelial disruption (E), subepithelial collagen deposition (SEC), myofibroblasts (M) and inflammatory cell infiltrates (IC) ($\times 1,060$).

dase A. The high content of neutral proteases in mast-cell granules implies important mediator functions. For example, tryptase can activate one of the protease-activated receptors (PAR-2) expressed on the surface of endothelial and epithelial cells. This leads to enhanced cytokine production, and the expression of adhesion molecules that selectively recruit eosinophils and basophils¹³. Although mast cells contribute to chronic airway inflammation, it is activated eosinophils that are considered to mediate most of the disordered airway function characteristic of this disease. Their selective recruitment and activation is a characteristic feature of asthma, whether allergic or non-allergic. Until recently, relatively little was known about how eosinophils released their mediators in allergic tissue responses. The recent recognition that, in the presence of IL-4 or IL-13, tissue but not circulating eosinophils express Fc ϵ R1 provides one mechanism whereby the local cytokine milieu is able to create allergen-responsive cells, while cleavage of eosinophil PAR-2 receptors by mast cell tryptase offers another.

Eosinophil recruitment from the circulation into a tissue subjected to an allergic response requires signals to reach the bone marrow. IL-5 is highly effective at releasing eosinophils and their precursors from the bone marrow¹⁴. Thus, in allergic asthma, both the circulating and sputum eosinophilia before and after allergen exposure were effectively suppressed by a single injection of a humanized blocking anti-IL-5 monoclonal antibody¹⁵. A similar effect has been observed with human recombinant IL-12, in this case operating by the suppression of IL-5 and other Th2 cytokine production by INF- γ (Fig 1). In contrast to similar experiments conducted in animal ‘models’ of

asthma, neither of these treatments affected the early or the late bronchoconstriction responses, nor *bronchial hyperresponsiveness* in humans. These highly selective interventions in allergic asthma not only question the interpretation of relatively acute animal models, but also the primacy of eosinophils as mediator-secreting cells in asthma. One interpretation for the findings is that reducing circulating and sputum eosinophils may not reflect a parallel reduction in primed eosinophils already resident in the airways, especially as in asthma protection of tissue eosinophils from apoptosis is at least as important as new cell recruitment¹⁶. In this regard, granulocyte-macrophage colony-stimulating factor (GM-CSF) produced by the epithelium and subepithelial myofibroblasts as well as eosinophil contact with matrix proteins are especially relevant.

Allergy: nature or nurture

Asthma clusters in families. The role of genetic factors as important determinants of allergic disease is revealed in studies of mono- and di-zygotic twins and in highly inbred populations such as those living in Tristan da Cunha where asthma affects up to 40 per cent of the population. Genetic factors operate at two levels determining: (i) allergen recognition through MHC class II haplotypes; and (ii) the organ localization and response. Although genetic factors may explain at least part of the wide intercountry differences in disease incidence, they do not explain the rising disease trends. The hypothesis that currently finds most favour is the influence of hygiene in western society that deprives the developing immune response of important signals for Th1 development, especially in children born of atopic parents and who at birth exhibit impaired IFN- γ production¹⁷.

The hygiene hypothesis best accommodates the link between allergy and social class, the urban to rural gradient, infant diet, over-use of antibiotics and the East to West gradient of disease. It involves the concept of faulty programming of the mucosal immune system *in utero* and during a critical period of childhood up to five years of age. However, these environmental influences best fit the rising trends in *atopy*, rather than asthma. Since the reunification of Germany in 1989, the prevalence of atopy in east Germany has almost caught up with that in the western half of the country and yet the prevalence of asthma has not changed. Similarly, over the past two decades, atopy has increased markedly in Nigeria, Ethiopia and the Gambia, but the prevalence of asthma and bronchial hyperresponsiveness has failed to parallel this. It would seem that factors other than atopy are required for the expression of the more chronic allergic disorders and that they are likely to have their origin in the organ itself as revealed in asthma by lung

transplantation¹⁸.

In all types of asthma, fragility and activation of the bronchial epithelium, proliferation of the underlying myofibroblasts and the laying down of 'repair' collagens (types I, III and V) in the *lamina reticularis*, which causes thickening of the sub-basement membrane region, are as characteristic of the disease as the presence of activated mast cells and eosinophils (Fig. 2). These unique pathological features of asthma indicate strongly that reactivation of the epithelial-mesenchymal trophic unit occurs which, in the fetus, is involved intimately in lung growth and branching¹⁹. In asthma, the same morphogenetic signalling molecules, including the epidermal growth factor family, transforming growth factors, basic and acidic fibroblast growth factors, nerve growth factors, vascular endothelial growth factor and keratinocyte growth factor and their respective receptors, are either over-expressed or exhibit evidence of activation. This suggests that epithelial-mesenchymal signalling is enhanced, and may account for some of the airways smooth muscle hypertrophy and other features of the remodelling of the airway wall in this disease²⁰.

Key questions to answer are whether epithelial-mesenchymal signalling is a primary or acquired abnormality and how these morphogenetic changes interface with the Th2 cytokine response. Epithelial cells and fibroblasts express receptors for IL-4 and IL-13 as well as signal transducer and activator of transcription 6 (Stat-6) and insulin-receptor substrates (IRS-1 and IRS-2) and respond to these Th2 cytokines with phenotypic responses that are compatible with observations in asthma (for example, *goblet cell metaplasia*^{21,22}, the enhanced secretion of the eosinophil chemoattractant eotaxin, and differentiation of fibroblasts to myofibroblasts²³). Functional *polymorphisms* of the relevant genes will not only impact on Th2 and IgE responses but will also influence the organ phenotype upon which these immune responses act.

Conclusions

In this supplement, the up-to-date set of reviews by a group of international experts covers the spectrum of knowledge of allergy and asthma, from epidemiology to molecular mechanisms and future therapies. The recognition that most atopic disorders have their origins in childhood, are increasing in incidence in developed and developing countries and have strong links to the environment suggests that solutions to stop the epidemic are more likely to come from public health than pharmacological interventions²². The challenge for the new century is to understand how environmental factors interact with the human genome to reveal the atopic state, its organ specificity and the associated diseases.

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The alliance of genes and environment in asthma and allergy

William Cookson

The diseases of asthma, eczema and hay fever are typified by reactions to common allergens, which are mediated by immunoglobulin E. These allergic diseases are increasing in prevalence, and are now a major source of disability throughout the developed world. They are the result of complex interactions between largely unknown genetic and environmental mechanisms. The identification of the environmental factors offers the real possibility of prevention of disease, and unravelling the genetics of allergic illnesses is likely to change their classification and treatment. Early life seems particularly important, when the initiation of allergic disease may result from genetic and environmental modification of the immune interaction between mother and child.

Allergies in one form or another affect a large part of humanity. 'Allergy*' was originally intended to mean 'deviation from the original state' when vaccination and injection with proteins and sera were seen to lead to a variety of harmful immune reactions¹. The word '*atopy*' (meaning 'strange disease') was coined to differentiate the familial syndrome of *asthma* and hay fever from these mixed reactions². Infantile *eczema* has subsequently been recognized to be part of the same syndrome, and is often referred to as atopic dermatitis. Common usage has meant that the original broad meaning of allergy is lost, and the terms 'allergy' and 'atopy' are often used interchangeably, which has been the practice in this supplement.

Allergies are caused by the immune reaction to common inhaled proteins, known as *allergens*. Typical allergen sources include house dust mite, grass pollens and animal danders (sheddings from skin and fur). From a worldwide perspective it is the house dust mite that is of most importance to asthmatics. The total annual exposure of an individual to allergens is often only in the order of micrograms.

Allergens appear harmless in themselves, and only cause disease because of the intensity of the immune reaction which they provoke. This reaction is typified by *immunoglobulin E* (IgE) responses against the allergen. IgE binds to its high-affinity receptor (FcεRI) on *mast cells* and other effector cells. Exposure to allergen in a sensitized individual leads to crosslinking of IgE/FcεRI complexes, which causes mast-cell degranulation and the initiation of inflammation.

The synthesis of IgE is under complex control, which is discussed in detail by Corry & Kheradmand in this supplement. Interleukin (IL)-4 in particular is associated with upregulation of IgE, and interferon-γ (IFN-γ) with downregulation. The modulation and maintenance of inflammation may be carried out by mechanisms that are independent of the IgE/mast-cell axis.

The atopic state is recognized clinically by skin prick tests, in which allergen solutions are placed on the skin, and minute quantities of allergen are introduced into the epidermis by pricking with a needle through the solution. If IgE specific for the allergen is present, mast-cell degranulation takes place, with a resulting visible weal and flare (Fig. 1a). This early reaction resolves within tens of minutes, but it can be followed some hours afterwards with a late response, in which immune cells infiltrate into the skin. Both early and late responses are seen in the lungs and nose, when exposure to allergens induces the symptoms of asthma or hay fever. Many people who are atopic by skin tests do not have symptoms of allergic disease, indicating that factors other than the atopic state are important in causing asthma or eczema. *Anaphylaxis* is an extreme form of allergic reaction, in which systemic mast-cell degranulation

takes place, followed by intractable bronchospasm, cardiovascular collapse and even death.

Atopic individuals can also be recognized by the presence of allergen-specific IgE in their serum, by elevations of the total serum IgE, and by the presence of eosinophilia in their blood. These patterns are also seen in normal individuals who are suffering from parasitic infection, indicating that the processes of atopic inflammation evolved to deal with such infections.

Asthma and eczema

Asthma and eczema are the most serious of the allergic diseases. Asthma has become an epidemic, affecting 155 million individuals in the world. The cost of treating the disease is substantial. In the United States the figure approximates US\$6 billion dollars per annum³, in Germany 5 billion DM (US\$3 billion)⁴, and in Britain £1 billion (US\$1.6 billion)⁵. More than half of this expense is spent on hospital care and 80% of the entire bill is attributable to the 20% of patients who require the most treatment³. The market to the pharmaceutical industry for asthma medication is \$5.5 billion per annum⁶.

Asthma is an inflammatory disease of the airways of the lung (Fig. 1b). Narrowing occurs because of inflammation and mucous hypersecretion, and is exacerbated as the smooth musculature in the bronchiolar walls becomes hyperresponsive to nonspecific stimuli. Intermittent airway constriction gives rise to the asthmatic symptoms of wheeze, cough, chest tightness and shortness of breath. Over time the bronchioles may become fibrosed or scarred, and the airflow limitation may become permanent.

Asthma is unlikely to be a single disease. Although most childhood asthmatics are also atopic, there is a wide spectrum of severity with mild intermittent wheezing lumped diagnostically with severe intractable disease. Adult-onset asthma is a poorly defined illness, which is more common in women and difficult to treat, and which in many cases does not seem to be atopic. Cigarette smoking is often a contributing cause. Other asthma syndromes, such as aspirin-sensitive asthma and industrial asthma, may also operate through non-atopic mechanisms.

Eczema is as common as asthma, affecting between 10 and 20% of children in western populations^{7,8}. It is characterized by an itchy red rash that has an easily broken surface and is primarily a disease of early childhood. It has a predisposition for flexures in the skin, such as at the front of elbows or behind the knees, but in severe cases the rash becomes generalized. There is a very strong association between atopic dermatitis and asthma, so that 60% of children attending a clinic for eczema may also have the other disease⁹.

The nature of allergens

The respiratory organs deal with a multitude of airborne particles,

* Terms in *italic* are defined in the glossary on p. B39.

yet only a few of these particles from a limited number of sources consistently produce allergic reactions. Disease-associated allergens are usually soluble proteins. Most allergens have an enzymatic function in their natural state, but this is also true of most proteins, and a consistent activity shared by allergens has not been identified. Most allergens form particles of dimensions that allow them to become airborne and to penetrate the respiratory tree. Only a limited range of particle sizes are respirable, and are able to reach into the lung rather than being deposited in the nose and throat. It is still unclear whether the physical facts of allergens' ubiquity, respirability and solubility are sufficient to explain why they induce disease.

The allergens that most commonly produce reactions in susceptible individuals are known as 'major allergens'. Allergens are named according to their source and the order in which they were discovered. Major allergens from the house dust mite *Dermatophagoides pteronyssinus*, for example, include *Der p* I and *Der p* II. Other important major allergens are *Fel d* I from the cat *Felis domesticus*, *Bet v* I from pollen of the birch tree (*Betula verrucosa*) and *Phl p* I and *Phl p* V from the pollen of timothy grass *Phleum pratense*. Many other grass pollens, such as rye grass (*Lolium perenne*), have allergens that are similar to those of *Phleum pratense* (see ref. 10 for review).

Most common allergens have been extremely well characterized, with definition of the B- and T-cell antigenic determinants, and even the solution of the crystal structures (Fig. 2)^{10–12}. This intimate knowledge of structure means that the tools now exist for the complete description for the events controlling allergen reaction with the immune system.

Particular allergens are associated with different types of allergic illnesses. Grass pollen allergens cause hay fever, and allergens derived from house dust mites, cats and cockroaches cause

asthma¹⁰. This difference is probably due to the physical dimensions of the particles, as pollen particles are relatively large and do not penetrate low into the bronchial tree. Children with eczema do not show a consistent pattern of allergen sensitization, but react to many allergens, including those derived from eggs, milk and cheese as well as the common airborne allergens. Transient IgE responses to food proteins are seen to a lesser degree in most children¹³, so sensitization to food allergens may be a nonspecific marker of a heightened allergic propensity. How either food or respiratory allergens induce disease in the skin is problematic, but a simple physical solution might be that children with eczema have delicate skin, and that allergens dissolve in highest concentrations in the moist flexural areas that show predilection to disease.

The epidemic of asthma and atopic disease

Asthma was quite rare at the beginning of the century, but its prevalence in developed countries has now risen to true epidemic proportions. Although some of the rising prevalence is possibly spurious and due to improvements in diagnosis and increased reporting of symptoms¹⁴, the evidence for changes in objective measurements of asthma-associated phenotypes is overwhelming^{14–16}. The number of children with diagnosed atopic dermatitis has doubled in the past 20 years¹⁵, and hay fever has shown a similar escalation in prevalence.

This increase in all atopic diseases can only be due to a factor or factors in the environment. The presence of the increase has a message of importance: identification of the responsible factors is very likely to make asthma and atopy preventable.

In the popular imagination, asthma is the result of air pollution. There is no doubt that air pollution with ozone and particulates can exacerbate existing asthma and precipitate hospital admission. However, pollution in most westernized countries has declined dramatically at the same time that the prevalence of asthma has been increasing.

Perceptions of the role of pollution in asthma changed with the work of von Mutius and her colleagues, who, once Germany was reunified, compared the prevalence of asthma in schoolchildren from the cities of Munich and Leipzig. The level of pollution was far higher in Leipzig, and the researchers expected to find a concomitant increase in asthma frequency. Surprisingly, there was significantly less allergic disease in polluted Leipzig than in clean Munich¹⁷. Subsequent studies showed that atopy, measured by skin-test responses, was also less common in the east than in the west¹⁸. These findings have been mirrored by comparisons between other European populations¹⁹. In addition, the prevalence of atopy has risen steadily in east Germany as it has become more westernized²⁰,

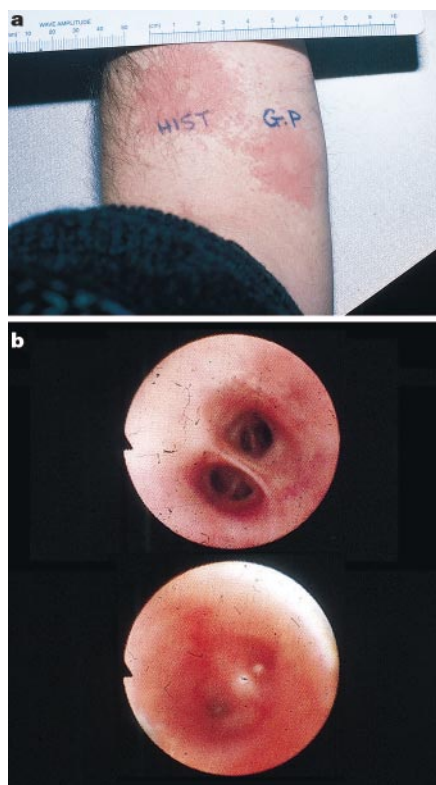


Figure 1 Consequences of allergic inflammation. **a**, Typical skin-test responses in an allergic subject, showing two wheals from grass pollen and histamine. **b**, Endoscopic appearances of an asthmatic airway, before and after local challenge with an allergen solution, showing inflammation in an asthmatic airway. (Courtesy of A. Frew.)

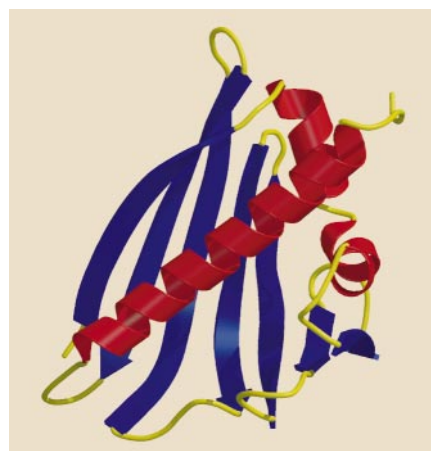


Figure 2 The crystal structure of the birch pollen allergen, *Bet v* I. (Courtesy of M. Gajhede.)

Box 1.

Epidemiological observations about asthma and allergy

- There are tenfold regional differences in the prevalence of asthma and atopic diseases
- Allergic disease is more common in clean westernized environments
- Asthma and atopy are less common in the children of animal farmers
- Asthma and atopy are less common in younger siblings
- Asthma and atopy are less common in households with dogs as pets

although an increase in the prevalence of asthma is not yet evident. The results suggest that the increase in the prevalence of allergic disease is due to factors associated with a western lifestyle.

International studies of children²¹ and adults²² have shown substantial regional differences in the prevalence of atopic disease, indicating that epidemiological studies may be able to identify the factors predisposing to disease (Box 1). Asthma, diagnosed by questionnaire, is most common in children from Britain, Australia, New Zealand and the Republic of Ireland, and lowest in eastern Europe, Indonesia, Greece, China, Taiwan, Uzbekistan, India and Ethiopia²¹. A rural lifestyle is protective both in Europe and Africa^{19,23,24}. Within Europe, the prevalence of atopic disease and atopic sensitization (measured by skin tests) is lowest in the children of farmers who keep animals near to the house^{23,25}. In non-farming households, the presence of a dog is also protective against disease²⁶. There is an effect of birth order, with the youngest children of large sibships also being protected²⁷, and the presence of an older brother being particularly effective²².

Events in early infancy are critical in determining the subsequent course of allergic disease. In the Scandinavian countries, a short intense spring flowering of birch trees is accompanied by symptoms in many individuals. Children born in the three months around the pollen season carry an increased risk of allergy to birch pollen for the rest of their life²⁸. In English children the level of house dust mite in infants' bedding during the first year of life correlates with the subsequent risk of childhood asthma²⁹.

The patterns of atopic disease could be explained by differences in the nature of childhood infection or changes in the bacterial flora of the bowel that follow from a clean western lifestyle^{17,19,30}. It has been argued that our immune systems need to be taught, in the same way as our nervous systems³¹. We have co-evolved with our parasites and the constant threat of bacterial and viral infections, and now that infancies in the westernized world are largely free from such companionship, 'immune deviation' and allergic and other diseases may be the result. The cellular arguments behind this hypothesis are developed in the subsequent review in this supplement by Holt *et al.*

Epidemiology is a branch of medical science which moves slowly but with great force. The power of the epidemiological approach is exemplified by an investigation of outbreaks of severe life-threatening asthma in Barcelona³². Twenty-six circumscribed outbreaks took place between 1981 and 1986, filling the city's hospitals and causing several deaths. An epidemiological investigation was set up, which after examining many possibilities recognized that the cases of asthma were clustered near to the Barcelona harbour. The investigators looked for correlations between the type of products unloaded from ships and days in which outbreaks of asthma took place. All asthma-epidemic days in the years that they tested had coincided with the unloading of soybeans. The investigators also found that the wind conditions favoured the movement of air from the harbour to the city on every epidemic day. Finally, they tracked the source of the soyabean dust to a single harbour silo that lacked bag filters at its top.

Although the worldwide increase in the prevalence of asthma and allergies may not eventually be attributable to such a well defined source, the rich and varied pattern of prevalence of atopic diseases

suggests that further epidemiological victories are possible.

The genetic basis of asthma and atopic disease

Asthma, eczema and the atopic state are strongly familial and have a genetic basis. Studies of twins have shown generally that concordance rates for asthma are significantly higher in monozygotic twins than dizygotic twins, and that the heritability of asthma may be as high as 75% (ref. 33). Analysis of the segregation of asthma within families indicates that asthma is influenced by a few genes with a moderate effect ('oligogenes'), rather than many genes of small effect ('polygenes')^{34–36}. Twin studies of eczema in children have shown concordance rates of 77% and 15% in monozygotic and dizygotic twins, respectively³⁷, indicating strong genetic effects on the disease. Parental eczema confers a higher risk of eczema in offspring than parental asthma or *rhinitis*³⁸, suggesting the presence of eczema-specific genes. Analysis of the inheritance of the total serum IgE, which is the principal marker of the atopic state, has also suggested the presence of genes of strong effect^{39,40}.

Given the likely presence of genes of strong effect, it is a reasonable expectation that understanding the genetics of both asthma and eczema will lead to improvements in their diagnosis, prevention and treatment. As a result, programmes aimed at the discovery of genes that predispose individuals to these illnesses are being carried out both within the industrial and the academic spheres.

Molecular genetics is about matching polymorphism in phenotype to polymorphism in the genetic material. Common diseases such as asthma are likely to be due to common variants ('alleles') in genes that alter gene functions in a subtle way rather than ablate gene function. This variation is as likely to be concentrated within the regulatory elements of the gene as within coding sequences.

Two general techniques are possible for identifying genetic effects on a disease. The first, known as the candidate gene approach, is to find polymorphism in a known gene and to compare the frequency of alleles in cases and controls. The second, and far more ambitious and expensive approach is that of positional cloning, which relies initially on linking the inheritance of specific chromosomal regions with the inheritance of disease⁴¹. Positional cloning has been extremely successful in identifying the genetic defects underlying single gene disorders, such as muscular dystrophy, cystic fibrosis and Huntington's disease. It is likely to make similar contributions to complex diseases such as asthma and atopy.

As a rule, academic groups are confined to the candidate gene approach because it is easier and cheaper, and industrial groups prefer positional cloning because it is systematic and because it has the ability to identify unexpected genes and mechanisms. In addition, the resources of industry allow the rational matching of the US\$5.5 billion annual market for asthma against the few million dollars required for gene discovery programmes.

Candidate gene studies

Although candidate gene studies may not find new genes, they still help the choice of targets for therapeutic intervention. Matching polymorphism in a candidate gene to a particular phenotype will demonstrate that interference with the action of this gene will produce a change in the phenotype. It will also show that the modulation of that gene's function is likely to be safe, because variation in this pathway has been encouraged in nature. The absence of polymorphism does not infer absence of a critical role in a particular pathway, but the corollary that conservation of response is important in evolutionary terms may be a warning against interference with a particular gene or pathway.

Several candidate genes have been implicated in allergic disease. The first effect to be recognized was the human leukocyte antigen (HLA) class II restriction of the response to particular allergens (Fig. 3). The strongest and most consistent association is between a minor component of ragweed antigen (*Amb a V*) and HLA-DR2. Many other possible positive and negative associations of the major

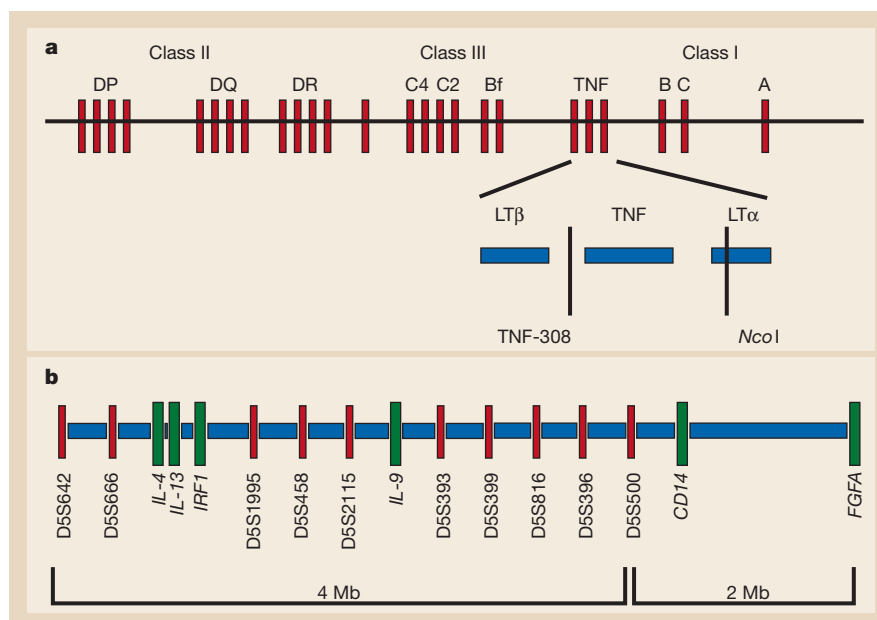


Figure 3 Gene clusters affecting allergic inflammation. **a**, The human major histocompatibility complex. HLA class II genes influence the ability to react to particular allergens, and TNF genes may modulate the intensity of airway inflammation independently of IgE responses. **b**, The cytokine cluster on chromosome 5. Polymorphisms are recognized in several of these genes (including those encoding IL-4,

IL-13, IRF1, IL-9, CD14 and FGFA), which may modulate the intensity of the allergic response. It is possible that genes within the cluster are co-regulated to give a consistent individual pattern of cytokine expression. D5S numbers refer to anonymous microsatellite markers used in the genetic mapping of the locus.

histocompatibility complex (MHC) with allergen reactivity have been described, most of which have not been confirmed (see ref. 10 for review). The overall effect of the MHC on allergens seems to be a multiplicity of small effects, usually conferring a risk factor less than twice as high as normal¹⁰. These results are unlikely to be useful clinically. Stronger HLA effects may be seen when the antigen is small and contains a single or very few antigenic determinants. This may be the case with aspirin-induced asthma and DPB1*0301, and sensitivity to inhaled acid anhydrides and HLA-DR3 (ref. 10).

Linkage of atopy to a genetic marker on the long arm (q) of chromosome 11 at band 13 (11q13) was reported ten years ago⁴², and was followed by a protracted argument about the significance of results⁴³. The experience gained from the investigation of this particular locus illustrates how the problems of sample size and selection, replication of genetic linkage, parent of origin effects (discussed below), genetic heterogeneity, and phenotypic pleiotropy must be solved for the effective and systematic identification of the genes predisposing to the atopic disorders.

The beta chain of the high-affinity receptor for IgE (*FcεRI-β*) was subsequently localized to the same region, and polymorphism with the gene related in different studies to atopy⁴⁴, asthma⁴⁵, bronchial hyperresponsiveness⁴⁶ and severe atopic dermatitis⁹. Polymorphism within the gene has also been associated with levels of IgE in heavily parasitized Australian aborigines, implying a protective role for the gene in helminth infestation⁴⁷. Although a few coding changes have been identified within *FcεRI-β*^{45,48}, they are conservative and do not seem to alter gene function.

The *FcεRI* receptor acts as the allergic trigger on mast and other cells, and is central to the allergic response. Its biology is described in detail by Turner & Kinet in the supplement. The β-chain is not essential for *FcεRI* function, but it both augments the surface expression of the receptor, and acts as an amplifying element within it. Any variation in the level of the β-chain expression may therefore modify receptor function. The controlling elements of the gene are now under study for genetic polymorphism.

Several associations have been noted between measures of atopy

and genes of the chromosome 5 cytokine cluster, including *IL-4* and *CD14* (refs 49, 50; Fig. 3). The congregation of cytokine genes in the region may have evolved for their co-regulation, and claims for the importance of particular polymorphisms within the cluster should be interpreted in the context of linkage disequilibrium with other markers and other known or unknown genes. An effect on atopy and serum IgE levels of *IL-4* receptor (on chromosome 16) has been recognized⁵¹, and may be stronger than the effects of polymorphism in *IL-4* itself. Coding variants within the β-adrenergic receptor have been shown *in vitro* to be functionally important⁵², but associations with asthma have been weak and inconsistent⁵³.

Tumour necrosis factor (TNF) is a pro-inflammatory cytokine that is abundant in asthmatic airways. An association between *TNF* alleles and asthma has been observed⁵⁴ and seems independent of the serum IgE or other measures of atopy, illustrating that asthma is not the result of only allergic mechanisms⁵⁴.

In general, these studies of candidate genes have been done on limited sample sizes. The next step in defining the utility of genetic polymorphisms in the clinical classification of asthma and allergies must be their application to large-scale epidemiological studies. It is also desirable to test sub-groups of allergic disease, such as children with severe illness, or illness that is resistant to treatment. In this context, the association of *FcεRI-β* polymorphisms with severe atopy, asthma, and eczema^{9,48,55} may eventually define a syndrome of early-onset severe allergic disease.

Pharmacogenetics

Individuals vary widely in their response to treatments with drugs. At present, optimal doses that balance side effects against therapeutic actions in a particular patient can often be determined only by trial and error. The study of pharmacogenetics may be able to use genetic polymorphism to predict the response of individuals to particular treatments. In the case of asthma, a variant within the promoter of the 5-lipoxygenase gene has recently been suggested to predict the response to the antileukotriene ABT-761 (ref. 56). A *Leukotriene C4 synthase* promoter polymorphism has been shown

Table 1 Genetic loci affecting asthma-associated phenotypes

Chromosome	Candidate genes	Authors
2	<i>IL-1, IL-1-RA</i>	71–73
5	<i>IL-4, IL-13, IL-9</i>	62, 63, 73
6p	MHC	67, 70, 71
12q	IFN- γ	64, 65, 70
13q		67–70

The loci presented in the table are those that have been identified consistently by genome screens and genetic-linkage studies. Localizations in many regions are only approximate, and the regions of linkage may cover large areas. Definite co-localization awaits typing with the same markers in all panels of families.

to be associated with increased risk of aspirin-induced asthma, and may also predict the response to treatment⁵⁷. It also seems possible that the polymorphisms in the β -adrenergic receptor regulate the response to the stock asthma treatment of β -adrenergic agonists. These results require confirmation and considerable extension, but may mark the beginning of the clinical use of genotyping as an adjunct to pharmacotherapy for asthma and many other disorders.

Positional cloning studies

Positional cloning is based on the study of families in which a particular disease or phenotype is transmitted to (or 'segregates' through) family members. The families are studied by visualizing nonspecific genetic polymorphisms that are in known positions on the chromosomes. These 'markers' typically contain repeat sequences of DNA, such as (CA)_n, which vary greatly between individuals and can differentiate between individual chromosomes in a family. Many thousands of such markers have been identified and integrated into a dense map that covers all chromosomes⁵⁸.

Once markers have been typed, the families are examined to see if any of the markers is inherited in the same way as the phenotype of interest. When marker and phenotype are co-inherited, genetic linkage is said to exist. The decision about whether genetic linkage is real or not is highly statistical, and made more difficult when hundreds of markers and several phenotypes have been tested. Stringent criteria need to be applied in these circumstances, so that if a genome-wide search for linkage has been carried out, a probability of less than 0.0007 may only be 'suggestive' of linkage, and a significant linkage may require a probability less than 0.00002 (ref. 59).

The discovery of genetic linkage usually means that a broad chromosomal region is likely to contain a gene influencing the disease phenotype. Saturation of the region with more closely spaced markers may reduce the interval for localization to between 5 and 30 million base pairs of DNA. Regions of this size will contain far too many genes to be studied in detail for effects on disease.

Happily, distinctive alleles of individual polymorphisms show non-random association with alleles of neighbouring polymorphisms in the DNA. This phenomenon, known as linkage disequilibrium, extends for far shorter distances than genetic linkage, and close localization of disease genes is likely to depend on the detection of linkage disequilibrium between marker alleles and disease phenotypes. The remaining intervals of 0.5 to 1.0 megabases can then be dissected systematically.

The choice of phenotype is critical in determining the success of positional cloning. *Atopic asthma* is easily recognized clinically, and has an obvious familial clustering. For this reason, many genetic studies have concentrated on the asthma of childhood, and the underlying atopic state. The simplest type of genetic linkage study relies on families that contain two affected children. The assumption behind this approach is that environmental factors will have acted on both siblings, and may be discounted in the statistical analyses of data. Unfortunately, the high frequency of asthma and atopy in the population has adverse effects on the power to detect linkage to affected sibling pairs⁶⁰. Simulation studies indicate that

Table 2 Coincidence of loci affecting asthma-associated phenotypes and other disorders

Chromosome	Candidate genes	Diseases
Chromosome 2	IL-1 cluster	Inflammatory bowel disease, Rheumatoid arthritis
Chromosome 6	MHC	Multiple
Chromosome 7		Inflammatory bowel disease
Chromosome 11		Type I diabetes
Chromosome 12	IFN- γ	Inflammatory bowel disease
Chromosome 14	TCR- α	Rheumatoid arthritis
Chromosome 16		Inflammatory bowel disease

500 affected sibling pairs will give less than 50% power to detect linkage to a given marker, and that over 1,000 affected pairs will be required to achieve 90% power. The use of quantitative traits underlying asthma together with selection for phenotypic discordance within families may be important if studies are to detect linkage with sufficient power to generate consistent findings^{60,61}.

Despite these difficulties, several genetic regions have been identified consistently by different groups (Table 1). Regions studied because they contain important candidates include chromosome 5q (containing the *IL-4/IL-9* interleukin cluster)^{62,63} chromosome 12q (near the *IFN* gene)^{64,65} and chromosome 14q (near the *TCR- α / δ* cluster)⁶⁶.

Several genome-wide searches have been carried out. A search for quantitative traits underlying asthma identified significant evidence for linkage on chromosomes 4q, 6 (near the MHC), 7, 11q (containing *Fc ϵ RI- β*), 13q and 16 (ref. 67). A replication sample of families in the same study confirmed linkage to chromosomes 4, 11, 13 and 16 (ref. 67). Linkage of the total serum IgE to a protein polymorphism on chromosome 13 had been identified several years earlier⁶⁸, and linkage to the region has been again confirmed by a single locus study of Japanese families⁶⁹. The same study identified potential linkage disequilibrium between disease and a particular marker⁶⁹. A two-stage screen in Hutterite families from the United States found evidence for linkage in several chromosome regions, with replication for loci on chromosomes 5q, 12q, 19q and 21q (ref. 70). Linkage to 13q was seen in the first panel of families only. A screen in German families identified evidence for linkage to asthma on chromosomes 2q, 6p (near the MHC), 9 and 12q (ref. 71). A genome screen for responsiveness to house dust mite allergen found suggestive linkage to chromosomes 2q, 6p (MHC) and 13q, as well as chromosome 8p (ref. 72). A genome screen in American families from three racial groups found possible linkage to the same regions as other studies on chromosomes 2q, 5q, 6p, 12q, 13q and 14 q (ref. 73). Several other possible loci were identified, but have not yet been confirmed. A two-stage genome screen in French families found replicated linkage on chromosomes 1p, 12q and 17q (ref. 74).

Genome screens have also been done by several industrial groups⁶, the results of which remain secret. The replication of linkage results is necessary before committing to identifying genes across a large region. In these circumstances, the industrial instinct of secrecy may be counter-productive.

The data from all these studies indicate that chromosomes 2q, 5q, 6q, 12q and 13q contain loci that consistently affect asthma and atopy with sufficient strength to be detected by genetic linkage. The challenge is to distil all this data and to refine the localizations sufficiently for gene identification to begin. This process would be helped by agreement between groups to saturate these regions with the same panels of markers, and to allow meta-analysis of the pooled data. Progress towards this goal has been taken by the agreement of some groups to pool data from chromosome 5 in the Consortium on Asthma Genetics (COAG) study.

Genetic studies of other disorders may also have an impact on asthma and atopy (Table 2). Crohn's disease and ulcerative colitis are inflammatory bowel diseases of unknown aetiology which show



familial clustering. Genome-wide screens have implicated loci on chromosomes 3, 7, 12 and 16 (refs 75–77). The regions on chromosomes 7, 12 and 16 coincide closely with the asthma and atopy loci on the same chromosomes. Polymorphism in the *IL-1* cluster on chromosome 2 has also been shown to influence the severity of inflammatory bowel disease⁷⁸. A genome-wide screen in families with rheumatoid arthritis has similarly shown linkage near the asthma locus on chromosome 2 and the *TCR- α* locus on chromosome 14 (ref. 79). Linkage to type I diabetes is found near *Fc ϵ RI- β* on chromosome 11q13 (ref. 80). These findings indicate that important genes or gene families may be common to several inflammatory and immune disorders.

The practice of genetics is changing rapidly. The genome will be largely sequenced within a year, so that the painstaking construction of physical maps of individual regions will no longer be needed. The technology that allows the expression of thousands of genes to be measured simultaneously⁸¹ allows the facile observation that the expression of many genes differs between asthmatics and non-asthmatics. The sheer volume of nonspecific genes activated in any cellular process may limit the usefulness of this approach.

Genome-wide association studies that use the typing of thousands of single nucleotide polymorphisms (SNPs) have been proposed as a more powerful method of detecting genetic effects than linkage mapping⁸². This approach would seem to bring considerable difficulties of statistical analysis, if only for the need to apply a correction of more than 100,000 for multiple comparisons to each data point. Assumptions about the extent of linkage disequilibrium through the genome have yet to be tested in real data, and the effects of allele frequency on detection of linkage disequilibrium to disease alleles has not been investigated. Nevertheless, it is quite possible that expression studies and SNP mapping may form the bridge between localization to large regions and the identification of disease genes and their function.

Maternal effects on asthma and allergic disease

Many epidemiological studies have indicated that maternal phenotype influences the inheritance of asthma and allergy (ref. 83 for review), although in general the studies were not designed specifically to test for parent-of-origin effects. The presence of asthma, eczema, elevated serum IgE levels and positive skin-prick tests in children have all been accompanied by an increased prevalence of asthma or atopy in mothers⁸³. The differential risk of transmission between parents is approximately fourfold. Parent-of-origin effects have been noted in other immunological disorders, most notably type I diabetes⁸⁴, rheumatoid arthritis⁸⁵, inflammatory bowel disease⁸⁶ and selective IgA deficiency⁸⁷, indicating that parental effects on the developing infant's immune system may be an important common process.

The immune systems of the maternal–fetal unit operate in a narrow space between rejection and nurture, in which proteins derived from paternal genes may be differentially antigenic to those derived from maternal alleles. Two types of maternally driven mechanisms may possibly operate to control or modify the fetal immune system. First, the maternal effect might result from immune interactions between the fetus and the mother. Maternal–fetal immune interaction is well recognized, and takes place through the placenta as well as through breast milk (see review by Holt *et al.*, this supplement). This interaction may have profound effects on the developing fetal immune system. Second, the maternal effect may be the result of genomic imprinting. Genomic imprinting is a process in which the allele from one parent is differentially expressed relative to the allele derived from the other parent⁸⁸. Imprinting seems important when there is a conflict of interest between maternal and paternal genes, such as those that control fetal growth.

A window to the investigation of maternal effects may come from the observation that linkage of severe atopy to the *Fc ϵ RI- β* locus on

chromosome 11q13 has been characterized by a maternal pattern, with linkage and association seen most strongly to maternally derived alleles⁸⁹. A recent family-based study of association between the alleles of an *Fc ϵ RI- β* SNP and atopic eczema showed a highly significant transmission of one of the maternal alleles, with an intriguing suggestion that the same allele was protective when transmitted from the father⁹.

Genomic imprinting would lead in an unselected population to preferential maternal transmission of a disease-associated allele regardless of maternal phenotype. Investigation of the effects of parental phenotype on linkage of the total serum IgE to *Fc ϵ RI- β* has been done in families taken from a general population sample. Parents were classified as affected if their age- and sex-adjusted total serum IgE was above 70% of the population. Linkage to the locus was seen most strongly when the mother was unaffected, or when the father was affected⁹⁰, which is not consistent with simple genomic imprinting. A maternal pattern of linkage to atopy does not seem to be confined to *Fc ϵ RI- β* , and the phenomenon has been observed at loci on chromosomes 4 and 16 (ref. 67). These results also indicate that the maternal linkage is not due to a simple genetic mechanism at a single locus.

Parent-of-origin linkages operate in other disorders. Preferential linkage of paternal alleles has been observed for the insulin locus and type I diabetes⁹¹, and HLA alleles for selective IgA deficiency⁸⁷, indicating that the mechanisms responsible for these findings may be common to several disorders.

Mono-allelic expression has been identified for a number of cytokines⁹² and allelic exclusion is a feature of T-cell antigen receptor (TCR) alleles expressed in individual cells. These phenomena are probably the result of the need for individual cells to commit to a consistent pattern of cytokine production. So far there is no evidence that the expressed allele derives preferentially from one parent or another. It is possible however, that the bias to one parent or another may be incomplete, or that it is most marked at a critical period in development of the immune system.

The maternal effect therefore seems deserving of further study, as it will throw light on the pathogenesis of allergic disease, and could also show a great deal in general about the nature of immunological reactions between mother and child.

Conclusions

The mechanisms of asthma, eczema and atopy follow from interactions between a system that has evolved to deal with parasitic and other infections, and a limited number of ubiquitous airborne protein antigens. Allergic diseases are a feature of westernized societies, and possibly result from reductions in the nature and frequency of childhood infections. Progress is being made in defining the genetic basis of asthma and atopy, with the hope of better classification and new treatments of disease. The relevance of some genes and genetic regions have been established, several of which may be active in other inflammatory diseases. Events early in life seem critical, and the development of atopy is fostered by the mother's strained immunological relationship with the fetus. □

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The role of allergy in the development of asthma

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Recent studies have shown that initial sensitization to airborne environmental allergens occurs typically in early childhood, but subsequent progression to persistent atopic asthma, which may not manifest for several years, is restricted to only a subset of atopics. The key to establishing the link between atopy and asthma lies in the development of persistent inflammation in the airway wall, resulting in structural and functional changes in local tissues which are responsible for the symptoms of the disease. This review summarizes recent findings on the nature of the cellular and molecular mechanisms underlying this process, and addresses the issue of why the intensity and duration of these tissue-damaging responses in the airway wall apparently exceeds the critical threshold required for development of persistent asthma in only a minority of allergy sufferers.

Allergy* is acknowledged as a major risk factor for *asthma*, and inhalation challenge of *atopic* (allergic) asthmatics with specific *allergen* evokes a biphasic response comprising discrete acute- and late-phase reactions separated in time by several hours¹⁻³. The defining clinical feature of these two reactions is airflow obstruction, which is reversible over short periods of time either spontaneously or as a result of treatment, and which is believed to be the direct result of inflammation of the airway wall.

At the cellular epicentre of this process are CD4⁺ T-helper memory cells (see review by Corry & Kheradmand, this supplement). These produce an array of *cytokines* that directly or indirectly 'program' the leukocytes that are responsible ultimately for acute and chronic allergic inflammation in the airways. As illustrated in Fig. 1, the principal type 2 helper (*Th2*) cytokines implicated in this process include interleukin (IL)-4, which is required to drive production of allergen-specific *immunoglobulin E* (IgE)⁴, IL-3, which controls mast-cell and *basophil* development⁵, and IL-5 in conjunction with IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF), which regulate the *eosinophil* component of allergy^{5,6}. In addition, there is growing interest in the role of Th2-derived IL-9 in regulation of IgE production⁷ and mast-cell growth⁸, and in IL-13 in relation to airways hyperreactivity (see below).

At the effector end of this process, the acute-phase component of allergy represents classical immediate-type *hypersensitivity*, triggered through allergen-induced crosslinking of specific IgE antibody bound to *mast cells* through high-affinity receptors. These cells release a range of granule-associated preformed *mediators* which are responsible for the immediate symptoms of the acute allergic response (in this case, bronchoconstriction), and which contribute directly to some aspects (for example, oedema) of the late-phase reaction. In addition, mast cells release a variety of *chemokines* and cytokines which contribute to recruitment and activation of secondary effectors, in particular the eosinophils^{1,2} that are one of the hallmarks of the late phase of allergic reactions. This late-phase response is characterized by airway perivascular oedema, mucous plugging and the presence of activated Th2 cells which represent the principal source of the cytokines responsible for the sustained recruitment and activation of eosinophils. As the late response develops at challenge sites the overall inflammatory infiltrate may include, in addition to eosinophils, significant numbers of *monocytes*, *neutrophils* and platelets together with representatives from a variety of subpopulations of T cells other than Th2 cells¹.

Opinion on the relative importance of the contribution of mast cells to the late phase of the allergic response has been divided. But evidence from studies in mast-cell 'knock-in' mice, which has demonstrated that these cells can contribute to aerosol allergen-induced granulocyte recruitment in a murine asthma model⁹, has reawakened interest in this issue, in particular in the potential role of mast-cell-derived tumour necrosis factor (TNF)- α ¹⁰. Indirect evidence also suggests a role for mast-cell-derived TNF- α in human asthma³.

It is also now recognized that structural cells such as airway epithelial cells are important sources of mediators in asthma, in particular in chronic disease¹. As discussed below, the recruitment of these cell populations into the overall inflammatory response may be an important component of the chronic phase of *atopic asthma*.

Allergy to airborne *antigens* has become increasingly common in many countries during recent years. However, despite the fact that most of the offending 'allergens' are present continuously in the natural environment, particularly the indoor environment¹⁰, only a small proportion of allergic individuals manifest symptoms of asthma, indicative of persistent allergic inflammation in their airways. Why is progression from allergic sensitization to disease expression in the airways not automatic, when all the necessary elements would seem to be present in the allergic individual?

In seeking to identify the missing link(s) in this process, this review traces the development of atopic asthma from the initial sensitization of naive Th cells against environmental allergens, through to the ultimate expression of chronic allergen-driven Th2-mediated inflammation in the airway mucosa.

The sensitization phase of respiratory allergy

There is increasing interest in the concept that asthma may be preventable if the early events in the process, in particular those involved in initial sensitization of the immune system in atopics against airborne environmental allergens, can be circumvented or forestalled. This has led to an increase in studies focusing on the initiation of immune responses to these agents in immunologically naive humans.

Our perception of the mechanism(s) underlying the primary allergic sensitization process has changed radically over the past few years. Following rapidly on the heels of pioneering work on Th-cell heterogeneity in the mouse⁴, T-cell cloning studies revealed major variations in cytokine production by inhalant allergen-specific Th-memory cells within the human population, with atopics expressing a cytokine pattern similar to murine Th2 cells^{11,12} or Th0 cells manifesting a mixed cytokine profile^{13,14}, in contrast to the Th1-like profile typical of non-atopics. The key cytokines in the atopic

* Terms in italic font are defined in the glossary on p. B39.

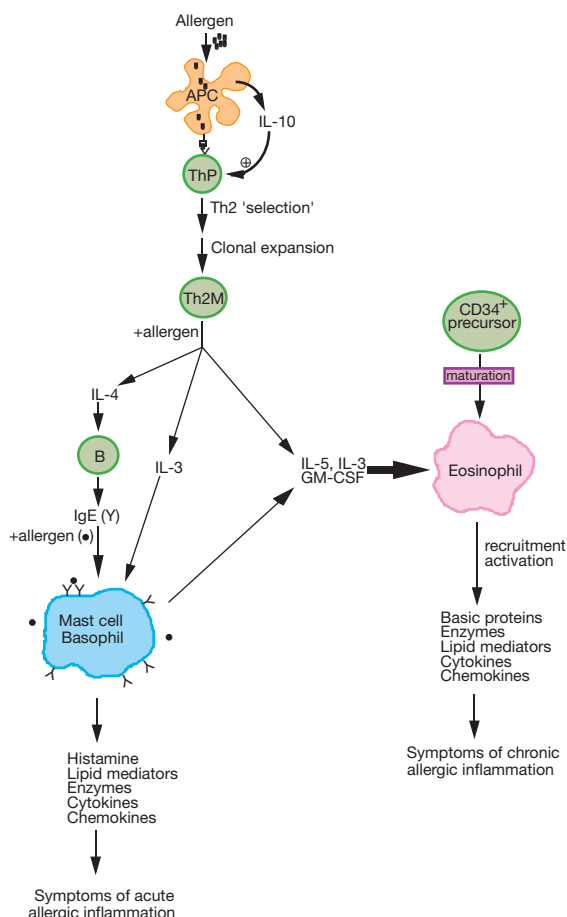


Figure 1 Major cell types implicated in the pathogenesis of acute and chronic allergic reactions. The initiation of this process involves presentation of processed allergen to naive Th-precursor (ThP) cells by antigen-presenting cells (APCs) within a cytokine milieu that favours the selective expansion of Th2-polarized memory cells (Th2M), resulting eventually in production of specific IgE by B cells. Re-exposure to allergen elicits an acute-phase response that is triggered through crosslinking of antibody-loaded high-affinity IgE receptors on mast cells/basophils. This is followed after several hours by a late-phase response involving inflammatory mediator production by Th2 cells and eosinophils with additional contributions from mast cells/basophils, and may also include contributions from macrophages, CD8⁺ T cells, neutrophils and platelets.

response seem to be IL-4 and IL-5, which drive IgE production and eosinophilia, respectively, after allergen exposure. The central issue in relation to the aetiology of allergy is thus how these disparate cytokine production patterns become programmed into long-term immunological memory.

The key events that determine the cytokine phenotype of allergen-specific Th-memory cells seems to occur very early in life, often many years in advance of the expression of persistent atopic disease. Surprisingly, initial priming of Th cells against environmental allergens commonly occurs *in utero*, presumably by means of transplacental transport of allergens to which the mother is exposed during pregnancy¹⁵. Paradoxically, these early allergen-specific Th-cell responses are dominated by production of the same Th2 cytokines that are associated with expression of atopy and asthma in later life¹⁵. This appears to be a direct result of selective downregulation at the fetomaternal interface of local production of Th1 cytokines, such as interferon (IFN)- γ , which are highly toxic towards the placenta¹⁶. The principal control mechanism in this process (reviewed in ref. 17) involves constitutive production by trophoblasts and other cells within the placenta of

prostaglandin E2, progesterone, IL-4 and IL-10, which are Th2-trophic and/or Th1-inhibitory.

However, beyond birth these Th2-trophic control mechanisms wane, and the weakly primed fetal Th-cell system is suddenly exposed to much higher concentrations of environmental antigens, and as a result fetal allergen-specific Th-cell responses are subjected to allergen-driven regulation. The emerging evidence indicates that high-level allergen exposure during infancy and early childhood, notably to dietary allergens such as egg albumen, leads to negative regulation of Th-cell responses^{18,19} via T-cell anergy and/or deletion¹⁷. In contrast, postnatal exposure to inhalant allergens, which occurs at much lower concentrations, results either in redirection of these responses towards a Th1-like cytokine pattern (in non-atopics)—a process termed immune deviation—or in further boosting of fetally primed Th2-polarized immunity in potential atopics^{17,19,20}.

The increasing tendency for failure of this allergen-driven immune deviation process during early life seems to lie at the core of the progressively rising prevalence of atopic disease among successive birth cohorts in the developed countries²¹. The cause of this failure is unclear, but some indications have emerged. First, it is apparent from our original studies²² and subsequent work of many laboratories (reviewed in ref. 17) that the rate of postnatal transition of Th-cell function from the Th2-skewed state characteristic of fetal life towards the relatively Th1-polarized adult state occurs significantly slower in children with genetic predisposition to atopy. We have hypothesized that this may compromise capacity to develop 'protective' Th1-like immunity against inhalant allergens during infancy^{17,21,22}. Second, much experimental literature indicates that the principal stimulus for normal postnatal maturation of Th1-associated functions is microbial exposure^{17,23}, which may partly explain some of the inverse relationships reported between childhood 'infections' and atopy development^{24,25}. It is of interest to note in this context the recently described association between a *polymorphism* in the gene encoding the high-affinity receptor for bacterial lipopolysaccharide (CD14) and intensity of allergic sensitization²⁶. This gene is located on the long arm of chromosome 5 adjacent to the IL-4/IL-9 gene cluster, and controls (*inter alia*) levels of soluble CD14, which in turn is central to the regulation of production of Th1-trophic IL-12 (ref. 26).

Clinical asthma and airways hyperresponsiveness

The term asthma describes a heterogeneous collection of clinical phenotypes as opposed to a single condition. A notable example is the distinction between the manifestation of the disease in children compared with adults. The point prevalence of asthma is actually greatest in young children, but up to half of these cease wheezing by adolescence²⁷. The most common trigger of asthma exacerbations (~85%) in children is virus infections, whereas infections account for only a minority of adult exacerbations²⁸. It is noteworthy, however, that children with the most severe asthma have symptoms which begin in early life, are likely to be clearly atopic, and do not tend to lose their symptoms in adolescence.

The key feature of persistent asthma is the development of the state of *airways hyperresponsiveness* (AHR). In the context of atopic asthma this equates to an exaggerated bronchoconstrictor response not only to allergens to which the subjects are sensitized, but also to a range of non-specific stimuli, including agents as diverse as cold air and methacholine.

How this hyperresponsive state is acquired is poorly understood. It is likely that AHR can result from several mechanisms, some or all of which may be operative in individual asthmatics. The result of chronic allergic inflammation in atopic asthma is airway remodelling (Fig. 2), involving thickening of the airway wall secondary to increased subepithelial collagen deposition (types III and V) and increased vascularity as well as hypertrophy and hyperplasia of airway smooth muscle (ASM)¹. In addition, increased numbers of

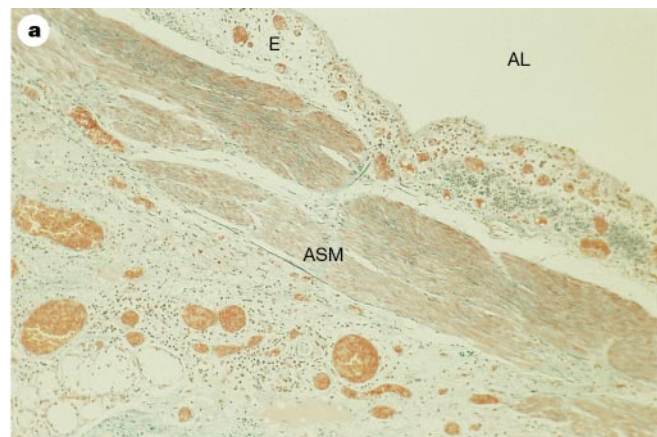
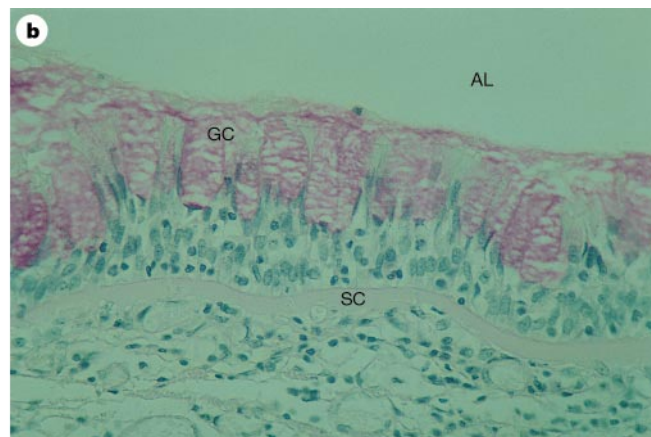


Figure 2 Transverse sections of the central airway from a subject with chronic asthma. **a**, Section stained with modified elastic trichrome, showing denudation of epithelium, enlarged muscle mass and prominent vessel dilation. AL, airway lumen; E, epithelium; ASM, airway smooth muscle. Magnification $\times 500$. **b**, Section stained with Periodic Acid



Schiffs reagent, showing goblet cell hyperplasia and collagen deposition below epithelial basement membrane. AL, airway lumen; GC, goblet cells; SC, Subepithelial collagen. Magnification $\times 1,000$. Images provided by N. Carroll, Sir Charles Gairdner Hospital, Perth.

goblet cells in the epithelium and alteration of autonomic neural input to the airways, especially the non-adrenergic non-cholinergic inhibitory and excitatory nervous systems, have been described.

Inflammation-induced airway remodelling may mediate AHR development through several discrete mechanisms, including those that alter neural regulation of ASM, increase ASM contractility, or that alter the sensitivity of ASM muscarinic receptors to cholinergic stimuli. In addition, airway remodelling may contribute to hyper-responsiveness through purely mechanical means. For example, an increase in the volume of tissue within the muscle layer—which may occur as a result of mucosal oedema, influx of inflammatory cells into the submucosa or increased vascularity of the epithelium—will result in greater airway narrowing and airway obstruction for a given degree of ASM shortening. Alternatively, an increase in the amount of ASM as a result of hypertrophy and hyperplasia will produce more shortening of ASM for a given stimulus and a greater increase in airway narrowing and airway obstruction. The combination of increased ASM and thickening of the airway wall would in theory produce an exaggerated airway narrowing response to environmental stimuli similar to that observed in asthma.

There are, however, additional mechanisms involving the lung parenchyma. In particular, the lung parenchyma is coupled to the airways so that when the lungs inflate, this mechanical interdependence results in the airways being pulled open. It has also been suggested²⁹ that the airways and parenchyma could be uncoupled by parenchymal inflammation and oedema, leading to changes in overall lung mechanics. Studies using transbronchial biopsies have indeed shown eosinophilic inflammation in alveolar walls in adult asthmatics³⁰. In addition, invasive studies that have used catheter-tipped manometers inserted into small airways of humans³¹, or that have directly measured alveolar pressure in animals³², have shown parenchymal responses to inhaled methacholine. Non-invasive techniques are being developed^{33,34} for studying lung parenchyma mechanics in humans in more detail and these promise to produce new information on the contribution of parenchymal lung inflammation to the overall changes in airway mechanics in asthma.

Although it is generally believed that airway remodelling in asthma is due to a disturbance in the balance of various growth and inhibitory factors within the airway mucosa, and thus secondary to chronic inflammation, our knowledge of the underlying cellular and molecular mechanisms is incomplete. For example, passive sensitization of ASM *in vitro* has been shown to alter muscle contractility. Although the process is dependent on the presence

of high levels of IgE antibodies, the mechanism is not understood with certainty³⁵. With respect to the deposition of interstitial collagens beneath the airway epithelial basement membrane during the remodelling process, there is compelling evidence implicating myofibroblasts as a major source^{36,37}, and recent *in vitro* studies indicate a potential stimulatory role for a variety of growth factors released from injured airway epithelial cells, including platelet-derived growth factor (PDGF), insulin-like growth factor (IGF)-1, basic fibroblast growth factor (bFGF) and transforming growth factor (TGF)- β ³⁸. But the relative contribution of individual growth factors to myofibroblast proliferation in this disease is still to be determined.

In vivo studies that seek to identify the precise aspect(s) of the atopic response that are responsible for AHR development and the attendant structural changes in the airway wall have only begun relatively recently, but some insight has been gained from animal models. In particular, experimental models have been developed in both mice^{39,40} and rats⁴¹. These demonstrate that CD4⁺ T cells that are transferred adoptively from sensitized to naive animals can induce AHR that is associated with IL-5 production and eosinophilia. Transgenic mouse models involving hyperexpression of individual cytokines in the airway epithelium are also providing insight into this process⁴², and into potential mechanism(s) underlying inflammation-induced deposition of extracellular matrix proteins in the airway wall^{43–45}. Receptor-blockade techniques are also proving useful in this regard, the prime example being studies demonstrating the importance of IL-13 in AHR development in mice^{46,47}.

Allergy and the development of clinical asthma

One of the least understood aspects of the pathogenesis of allergic disease is target-organ selectivity. So far, we have no satisfactory explanation for why apparently equivalent levels of hypersensitivity to the same allergens in different subjects can manifest in some exclusively as skin disease (atopic dermatitis), in others as nasal inflammation (*rhinitis*), while in others as multi-organ disease.

The situation with respect to asthma is particularly complex: although the vast majority of asthmatics are allergic to one or more inhalant allergens, only a subset of allergic subjects go on to ultimately develop persistent airway disease. A study in Australia has indicated that by early school age up to 40% of children display atopic sensitization to ≥ 1 inhalant allergen⁴⁸, and this figure remains stable into adulthood. However, despite the fact that $>90\%$ of adult asthmatics are atopic, it is evident from asthma

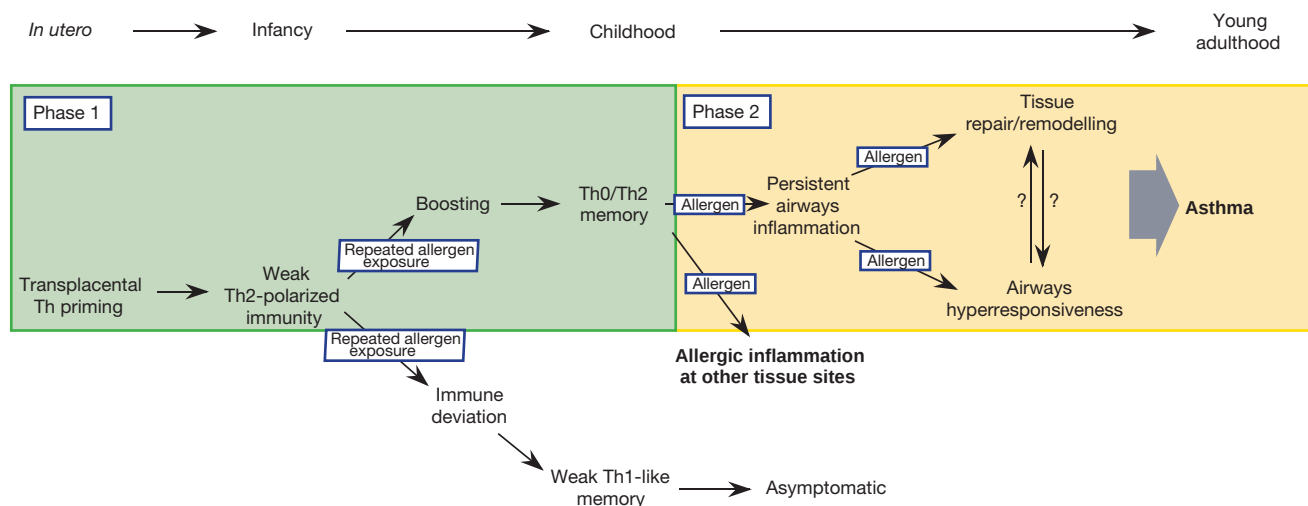


Figure 3 Progression from primary allergic sensitization in early childhood to atopic asthma in later life. Recent findings indicate that the development of asthma can be

considered to occur in two phases. Phase 1 involves T-cell 'sensitization' in early life, whereas chronic airways inflammation and its sequelae occur in stage 2.

prevalence figures in older children and adults that only 25–30% of sensitized atopics progress to asthma. On this basis it is reasonable to hypothesize that, in this subset of atopics, additional co-factor(s) must be operating which focus the disease process upon the airway mucosa.

As depicted in Fig. 3, the development of atopic asthma can be considered as a two-stage process. In the scheme shown, phase 1 involves the development of allergen-specific immunological memory against inhalant allergens. This process normally occurs during childhood, and in a subset of individuals it results in development of Th0/Th2-polarized immunological memory (that is, allergic sensitization), which increases 'risk' for allergic respiratory disease but is insufficient in itself for disease expression. The latter occurs only in those subjects whose allergic sensitization to airborne antigens results in persistent inflammation within the airway mucosa.

As noted above, the characteristic histopathological feature of atopic asthma is the presence in the airway mucosa of activated Th cells that produce Th2 cytokines. Repeated cycles of allergen-driven activation of these cells, resulting ultimately in a chronic 'wound healing/repair' response in these tissues, is believed to be central to the structural and functional changes in the airway wall that are characteristic of the asthmatic state.

Although yet to be proven, we can hypothesize that the level of Th2-mediated inflammatory damage sustained must exceed a certain critical threshold in order to precipitate long-term pathological changes in the airway wall, and thus progression to phase 2. Given this scenario, the most likely co-factor(s) that would operate in tandem with atopy in this second phase of the asthma disease process are those that can directly or indirectly add to the level of local airway inflammation. Possibilities for the latter include the following.

Exogenous airborne inflammatory stimuli. Current evidence indicates that the most important of these stimuli in quantitative terms are respiratory virus infections⁴⁹ followed by indoor levels of inhalant allergens to which the atopics are sensitized⁵⁰, both of which have been linked repeatedly to asthma exacerbations in children and adults. Additional possibilities include indoor irritants such as environmental tobacco smoke, and non-allergenic inflammatory particulates in house dust⁵¹, as well as outdoor air pollutants such as diesel exhaust components. Maternal smoking, both during pregnancy and postnatally, is associated with reduced infant lung function at birth and is a major independent risk factor for developing asthma⁵².

Failure of immunoregulatory mechanisms in airway mucosa. A series of overlapping control mechanisms serve collectively to limit the duration and intensity of T-cell mediated immunoinflammatory responses within the airway mucosa. Included among these is the local production of nitric oxide (NO), which can inhibit Th-cell activation through effects on T-cell signalling kinases⁵³. However, the T-cell inhibitory effects of NO are considerably more effective against Th1 than Th2 cells, and hence this mediator can potentially skew ongoing T-cell responses towards the Th2 cytokine phenotype via differential 'sparing' of the latter⁵⁴. NO is produced at high levels within the asthmatic airway⁵⁵.

Dendritic cells are the principal antigen-presenting cell (APC) population in the airway wall, but the functional phenotype of these cells is normally tightly controlled and is restricted to antigen uptake and processing^{56,57}. The cells require overnight exposure to GM-CSF to induce maturation of antigen-presentation functions⁵⁶. Although this cytokine is normally produced in only trace amounts in resting airway tissues, it is present locally at very high levels in atopic asthmatics⁵⁸. Recent evidence also suggests that these changes in cytokine production within the airway epithelium of atopic asthmatics are accompanied by increases in the local population density of intraepithelial dendritic cells^{59,60}, with concomitant upregulation of surface expression of activation markers⁶¹. Dendritic cells have been shown to have a key role in CD4⁺ Th-cell-induced experimental airway eosinophilia in an animal model of asthma⁶².

An additional control mechanism within the airway wall that may be disturbed in asthma involves the functions of local *macrophages*, which in the steady state downregulate T-cell responses via direct effects on T-cell cycling⁶³ and via inhibitory effects on APC⁵⁶. There is increasing evidence that monocytes recruited into inflammatory sites in the airways, including in asthma, express the alternative T-cell-trophic phenotype⁶⁴, and may thus contribute to persistence of the underlying immune responses which were responsible for their initial recruitment into the tissue.

Variation within Th2 responses. Considerable quantitative and qualitative variation is evident when allergen-specific Th2-like responses are compared between individual atopics⁶⁵, and some of these variations may contribute to the absolute level and tissue distribution of ongoing allergen-induced immunoinflammatory damage. Such variations may include allergen-specific T-cell clone size(s), and also the balance between crossregulating cytokines within individual responses, in particular the relative contribution of anti-inflammatory IL-10 (ref. 66). In this context, we have

recently shown that the magnitude of *in vivo* skin-test reactions in children to inhalant allergen is inversely related to the capacity of their T cells to produce IL-10 in response to *in vitro* challenge⁶⁷. An additional source of variation may be levels of surface expression on allergen-specific Th2 cells of mucosal 'homing' molecules (such as $\alpha 4\beta 7$; see ref. 68) and others affecting T-cell trafficking to specific tissues such as the cutaneous lymphocyte-associated antigen.

Recruitment of structural cells. The level of inflammatory cytokine production by structural cells in the airway wall in the absence of inflammatory stimulation is relatively low. However, in atopic asthmatics, airway epithelial cells, fibroblasts, endothelial cells and smooth muscle cells produce a wide range of cytokines and mediators¹, presumably as part of the tissue's response to chronic inflammation. These phenotypic changes in the airway mucosa may in part account for the persistence of airway inflammation in chronic disease sufferers, even in the apparent absence of ongoing exposure to inhalant allergens to which the subjects are sensitized.

Conclusions and speculation

Considerable progress has been achieved in recent years in identifying the many cellular and molecular effector mechanisms that contribute to acute- and late-phase allergic inflammation at challenge sites. The greatest impediment to the rapid translation of this growing information into development of more precisely targeted anti-inflammatory drugs is the redundancy inherent in most of these processes. However, a hierarchy of effector mechanisms is slowly becoming evident, particularly in relation to chronic allergic inflammation in which CD4⁺ Th-cell-mediated eosinophil participation has emerged as a key rate-limiting component of the disease process, in virtually all manifestations of allergy.

It is becoming increasingly clear that the development of severe allergic disease within individual tissues is in a sense an iterative process in which exogenously stimulated immunoinflammatory reactions provoke responses from the injured tissues (for example, 'repair'), which feed back to modulate the original immunoinflammatory response, and this altered response can in turn elicit further changes within the tissue. The chronically inflamed airway wall may represent the prime example of this process, with acute-phase allergy-induced inflammation providing the initial stimulus, which steadily intensifies as the contribution of the late-phase reaction increases with progressive expansion of the T-cell-driven component of the overall response. The reaction of the airway tissue to these insults includes a range of adaptive changes familiar to cell biologists with interests in 'wound healing', culminating eventually in structural changes that can have permanent effects on lung function. However, it is still not clear as to precisely which of these long-term changes within tissues of the airway wall are responsible for the individual symptoms of asthma.

It is also important to acknowledge that alternative immunoinflammatory pathways exist for asthma development. The best characterized examples are intrinsic asthma⁶⁹ and occupational asthma induced by toluene di-isocyanate⁷⁰. No role for IgE has been found in these diseases, despite the presence of cellular infiltrates and associated cytokine production in airway-wall biopsies which closely resemble the Th2-biased pattern that is typical of atopic asthma^{69,70}. It has been proposed that the underlying mechanisms driving these (and possibly other) forms of asthma may include airway epithelial-mediated deviation of all local immune responses towards the Th2 profile⁷¹, and this suggestion merits more detailed investigation.

Our current understanding of the relative contributions of these different types of mechanisms to disease expression in the airways is derived from extensive research that has concentrated on established (chronic) atopic asthma. An alternative approach to these complex issues, which is just starting to bear fruit, is to focus instead on the inductive phase of the disease process. As illustrated in Fig. 3, recent findings in the paediatric literature indicate that issues

associated with the development of allergy *per se* can be logically segregated from those associated with the expression of allergic inflammation in the airway wall, and in turn from the response of the target tissue to this repeated inflammatory stimulus. Placed within the appropriate developmental context, namely that these events occur typically over the period of life during which lung growth is maximal, this approach may provide a new perspective for finer dissection of the many interacting components of this complex disease process, and hopefully may shed new light on the factors that underlie the progression from acute to chronic disease. □

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Induction and regulation of the IgE response

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Immunoglobulin E (IgE) is believed to be one of the major mediators of immediate hypersensitivity reactions that underlie atopic conditions such as urticaria, seasonal allergy, asthma and anaphylaxis. Factors that control IgE production are therefore essential to the pathogenesis of these important afflictions. But a complete understanding of this topic is lacking, while new data have raised questions regarding the precise role of IgE in atopic disease. Evolving concepts of IgE production and elimination are likely to clarify the importance of IgE in health and disease.

The *atopic*^{*}, or *allergic*, conditions constitute a familiar group of diseases of rising importance in Western societies. Seasonal allergy, *urticaria* and *eczema* are believed to share a unique mechanism of disease which involves the binding of IgE antibodies to surface receptors present on a wide variety of cells, most importantly *mast cells* and *eosinophils* (Fig. 1). Crosslinking of receptor-bound IgE with *antigen* stimulates release of potentially toxic products that elicit the familiar signs and symptoms of atopic disease. These phenomena, called immediate or type I *hypersensitivity* reactions, include weal and flare eruptions of the skin (hives or urticaria) and sneezing, rhinorrhoea and conjunctival irritation (seasonal allergy). More serious conditions such as *asthma* (intermittent shortness of breath) and *anaphylaxis* (shortness of breath coupled with lowered blood pressure) are believed to share a similar pathogenesis. Although these diverse signs and symptoms are associated with disease, they originate from mechanisms that putatively serve a host-protective function. For example, IgE-dependent mechanisms may contribute to defence against immature parasites such as *schistosomula*¹ and may limit tissue injury induced by adult forms² (Fig. 2).

In many individuals, the total serum IgE level correlates roughly with both disease severity and protective immunity to parasites. However, even in patients with marked disease, with serum antibody levels in excess of 100 times normal, IgE titres rarely approach baseline levels of other antibody classes such as immunoglobulin-γ (IgG). Tight control of IgE may therefore be important to prevent potentially lethal consequences of IgE-dependent inflammation. Consequently, understanding the factors that control the serum IgE level becomes essential to dissecting the pathogenesis of atopic disease.

In this review we consider the complex regulation of the IgE response, distinguishing where possible the factors affecting antibody production and elimination. IgE production is intimately tied to subtypes of T cells and their *cytokine* products, certain pathogens, and the unique properties of the antigens, called *allergens*, associated with disease. Despite their equal importance in determining the serum IgE level, far less is known about the mechanisms of IgE elimination, although novel receptors at mucosal surfaces are probably involved.

Helper-T-cell subsets

The immune response is divided traditionally into two broad categories, humoral and cellular, emphasizing the distinct contributions of secreted antibodies and various effector cells, respectively. This dichotomy is increasingly difficult to maintain, however, as the essential cellular contributions to humoral immunity and humoral effects on the cellular response become apparent. Among the many

cells participating directly in immune responses, helper (CD4⁺) T cells have emerged as essential orchestrators of both humoral and cellular events, for example by providing the necessary signals for antibody production and through activation of cellular antimicrobial machinery. Helper T cells are further classified into two functionally distinct effector subtypes, a concept that is now a dominant paradigm in cellular immunology and that provides an elegant framework for understanding atopic inflammation and the IgE response³. In general, these effector subtypes are mutually antagonistic; consequently, inflammatory responses tend to be dominated by the effects of one cell type over another.

Th1 (type 1 helper) and *Th2* cells derive from a common precursor in response to distinct stimuli and are differentiated according to two major patterns of secreted proteins called cytokines (Fig. 2). *Th1* cells secrete interleukin (IL)-2, interferon (IFN)-γ, tumour necrosis factor (TNF)-α, lymphotoxin (LT) and other cytokines that together mobilize cellular and humoral defence mechanisms against intracellular pathogens and antagonize IgE responses. IL-2 and IFN-γ both suppress IgE synthesis through direct effects on B cells⁴⁻⁷. Although IL-12 and IL-18 are not type 1 cytokines in a strict sense, they also suppress IgE secretion, probably through induction of IFN-γ⁸.

In contrast, *Th2* cells secrete an entirely separate and functionally distinct repertoire of cytokines, including IL-4, IL-5, IL-6, IL-9 and IL-13. These cytokines seem to coordinate host defence against large, extracellular pathogens such as helminths⁹. Most of the characteristic features of atopy and asthma, especially IgE synthesis, result from the combined effects of type 2 cytokines and are closely associated with *Th2*-like cells^{10,11} (Fig. 2).

IL-4/IL-13 expression and signalling

IL-4, arguably the paradigmatic type 2 cytokine, is the most important cytokine mediating IgE synthesis^{6,12} (Fig. 3). Human¹³ and murine¹⁴ B cells also synthesize IgE in response to the closely related cytokine IL-13 (ref. 15). IgE synthesis has also been observed under unusual conditions in the absence of IL-4, but with uncertain relevance to atopic disease^{16,17}. Thus the biology of allergic disease closely parallels that of IL-4 and IL-13.

But the relevant sources of IL-4 required for IgE production remain unclear. The cellular sources of IL-4 are quite restricted, limited predominantly to T cells, although mast cells, *basophils*¹⁸ and *eosinophils*¹⁹ may also produce IL-4 and other cytokines. IL-13 shares a similar cellular distribution but is produced additionally by *natural killer (NK) cells*²⁰. In some experimental systems, *Th2* cells are alone sufficient to produce the IL-4 required for IgE synthesis²¹, but human *basophils* are able to elicit significant IgE production when cultured with B cells²². In addition, *eosinophils* are recruited early to sites of allergic challenge where they promptly secrete IL-4 in amounts sufficient for *Th2* cell outgrowth, thus establishing a

* Terms in italic font are defined in the glossary on p. B39.

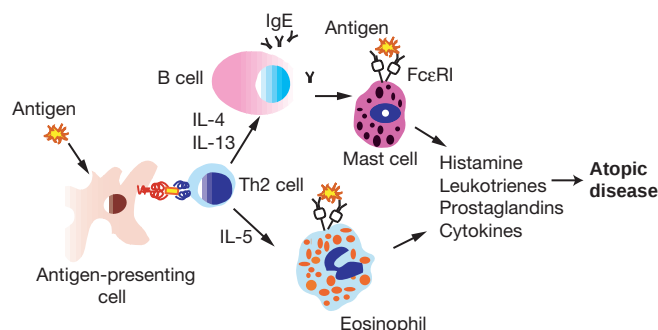


Figure 1 The type 1 hypersensitivity reaction. IgE produced by B cells is captured at the cell surface by Fc epsilon receptor I (FcεRI) present on mast cells and eosinophils. Cross linking of this receptor during subsequent encounter with antigen stimulates release of a variety of toxic products that together elicit atopic disease. The central role of the Th2 cell is evident: stimulated by antigen, these cells produce the IL-4 and IL-13 required for IgE synthesis and the IL-5 required for eosinophil growth and differentiation.

potentially important link between innate immunity and the IgE response²³. Natural T cells, CD4⁺ T cells expressing a restricted T-cell-receptor repertoire and the NKR-P1 surface antigen typically associated with NK cells²⁴ release sufficient IL-4 for IgE synthesis under some conditions but are not essential for the IgE response to allergens²⁵.

Recent studies have emphasized intracellular pathways in the molecular control of the IgE response through IL-4/IL-13. Although the composition of the complete IL-4 and IL-13 receptors is controversial, they share the alpha chain of the IL-4 receptor (IL-4Rα)²⁶. Engagement of this moiety with either ligand initiates a signalling cascade that results in translocation to the nucleus of signal transducer and activator of transcription 6 (Stat-6)²⁷ and initiation of germline epsilon messenger RNA (precursor mRNA for IgE) transcription and epsilon class switching (Fig. 3). The latter is a recombination event in which DNA encoding the antigen-binding domain of mature antibody is paired with the non-binding coding region that is specific for IgE. As determined by *in vivo* gene targeting, these events are dependent on IL-4Rα and Stat-6—mice that are genetically deficient in either molecule are incapable of IgE synthesis if given conventional antigen challenges^{28–30}. Consistent with these findings, individuals with gain of function mutations in both the extracytoplasmic and intracellular domains of IL-4Rα show enhanced IgE responses and predisposition to atopic disease^{31,32}. Further analysis of mutations in IL-4Rα and related genes, including their promoters and other regulatory regions, may identify additional patient subsets with dysregulation of IgE.

Other signalling pathways

Additional receptors and transcriptional regulators modulate the IgE response. The CD40 antigen is expressed on the B-cell surface after antigen recognition; engagement with its ligand (CD154; relative molecular mass 39,000) promotes IgE class switching, B-cell growth and other functions³³ (Fig. 3). Crosslinking of CD40 alone is sufficient to elicit a polyclonal IgE response, but when combined with signals from the B-cell antigen receptor the response becomes antigen specific¹⁶. As with IL-4Rα, complete deficiency of CD40 abrogates *in vivo* IgE responses^{33,34}. Nuclear factor kappa B (NF-κB) is the probable factor initiating CD40-dependent epsilon chain transcription and synergizes with Stat-6 to achieve maximal IgE production^{35–37}. Gamma (IgG) transcript synthesis is also initiated through CD40/NF-κB, but epsilon transcription is com-

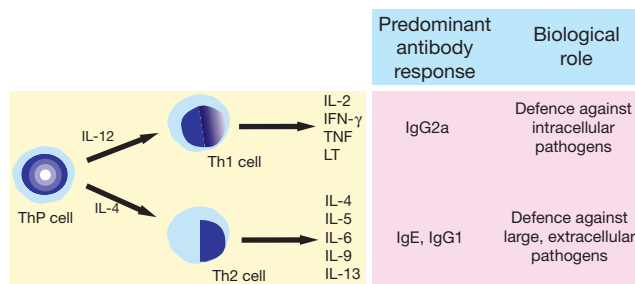


Figure 2 Overview of T-cell differentiation. Terminally differentiated helper T cells, termed T helper cells 1 and 2 (Th1 and Th2 cells), derive from a common precursor cell (ThP cell) depending on the signals received at the time of antigen recognition. Th1 cells emerge typically in the presence of IL-12, whereas Th2 cells are induced by IL-4. Upon re-exposure to antigen, Th1 and Th2 cells secrete distinct patterns of cytokines that serve to activate separate immune compartments. Th1 cytokines (IL-2, IFN-γ, TNF and LT) elicit antibodies (IgG2a) and other cellular responses that together promote defence against organisms entering the intracellular compartment. In contrast, Th2 cytokines (IL-4, IL-5, IL-6, IL-9 and IL-13) promote other antibody classes (IgE, IgG1) and cellular mechanisms that protect against larger organisms that cannot be taken intracellularly.

paratively low with respect to gamma transcription, in keeping with relative serum levels of the respective antibodies. Thus, additional mechanisms may exist that limit CD40-dependent epsilon transcription, perhaps representing an important means for controlling the serum IgE level³⁸. Other epsilon-specific transcription factors have been described, including C/EBP family members³⁹ and BSAP/pax-5 (ref. 40) but their *in vivo* relevance has yet to be determined.

Additional molecules expressed on the B-cell surface after encounter with antigen include CD80 and CD86 (formerly B7-1 and B7-2; Fig. 3). These co-stimulatory ligands engage the CD28 receptor present on T cells, providing an additional activation signal that is crucial to T-cell survival and cytokine secretion⁴¹ (Fig. 3). At least under some conditions, CD86 appears to be more important for Th2 cell development, IgE production and related allergic phenomena^{42,43}.

Nature of the antigen

It is now clear that the biochemical properties of antigens significantly influence the direction (Th1 or Th2) of the immune response and the rise, if any, of serum IgE titres. By definition, allergens, including products of some infectious organisms such as fungi and other parasites, give rise to Th2 cells and high serum IgE levels. In contrast, other antigens, including products of bacteria, do not produce a significant rise in IgE titres but instead elicit reactivity of other antibody classes and no or few Th2 cells. Such bacteria include *Listeria monocytogenes*⁴⁴, the agent of human listeriosis, and *Mycobacterium tuberculosis*, the agent of human tuberculosis. The mammalian immune system has evolved to recognize unique substances present in these organisms that influence the direction the immune response takes without necessarily being the target of T and B cells (that is, the antigen). Both listeria and mycobacteria contain conserved DNA sequences consisting of repeated cytosine and guanosine residues (CpG repeats) that are uncommon in eukaryotic DNA⁴⁵. These sequences are recognized by receptors on antigen-presenting cells (APCs) and trigger the release of IL-12, which suppresses IgE synthesis and attenuates the experimental asthma phenotype in mice^{8,46,47}. Components of the cell walls of these and related organisms may have a similar influence on APCs^{48,49}.

By analogy, it is likely that substances derived from fungi, parasites and other allergens direct the immune response to give rise to Th2 cells, IgE and thus atopy. Many allergens are enzymatically active, including *Der p* I (derived from *Dermatophagoides*

pteronysinus, the common dust mite), an allergen strongly associated with childhood asthma⁵⁰. This protease selectively cleaves surface CD23 of murine B cells, potentially interrupting an important negative regulator of IgE production⁵¹ (see Box 1). Additional mechanisms by which proteolytically active allergens may augment atopic disease have been suggested⁵².

The genetic association of atopy and certain human leukocyte antigens (HLAs) indicates another feature of allergens that may predispose to the development of atopy. Under otherwise neutral conditions *in vitro*, murine Th2 cells develop from precursor cells according to the strength of signals received from APCs. If the signal is extremely low or high, Th2 cells tend to develop whereas intermediate signals tend to produce Th1 cells^{53,54}. An essential component of this signal is received when class II major histocompatibility complex (MHC) molecules (analogous to HLAs in humans) interact with the antigen receptor of CD4⁺ T cells (Figs 1, 3). Allergenic peptides with low or high affinity for MHC molecules would be expected to confer relatively weak or strong signals through the same T-cell receptor, respectively, facilitating Th2 cell outgrowth and thus atopy.

Allergen exposure history

Similarly, peptides in very low abundance, which is typical of many allergens, would be expected to load incompletely into MHC molecules regardless of binding affinity. Weak presentation of peptides on this basis may also bias T-cell development towards IL-4 production, and thus atopy.

The route of exposure to allergen might also influence the magnitude of the IgE titre and its stability with time. Evidence indicates that antigen administered through the respiratory route is highly immunogenic, with a propensity to induce allergic

inflammation. In contrast, antigen encountered through other routes, especially intravenously and orally, is more likely to induce immunologic unresponsiveness or *tolerance*, the latter condition being a state in which the immune system fails to respond (or responds only transiently) to potentially recognizable antigen and cannot be induced to respond on subsequent challenge. The factors that make the respiratory immune response less susceptible to tolerance induction are not entirely clear. Immune surveillance is very efficient in the lungs, and necessarily so given their importance as barriers against infections. APCs, particularly *dendritic cells* (also called *Langerhans cells* in the skin), are a critical part of this defence mechanism. These 'professional' APCs are potent inducers of immunity through their array of surface molecules (including CD80, CD86 and MHC class II molecules) and secreted substances (including IL-12) that influence both the strength and the direction of the resulting inflammatory response⁵⁵. Recent studies demonstrate that airway dendritic cells underlie the Th2 polarity exhibited in airway immune responses^{56,57} and may induce Th2 cell development through a new pathway that does not involve IL-4 (ref. 58).

IgE receptors and binding factors

The regulation of IgE is associated intimately with specific receptors and other binding factors. CD23 seems to be the most important receptor affecting IgE production, while additional receptors at mucosal surfaces, so far poorly described, may be responsible for elimination of most IgE.

FcεRI. Although essential to the biology of IgE-mediated disease, the role of the high-affinity receptor for IgE, FcεRI, in the regulation of serum IgE levels seems to be minimal. FcεRI binds IgE at very low concentrations ($K_a = 10^9 \text{ M}^{-1}$), greatly prolonging the *in vivo* half-life of IgE⁵⁹. Furthermore, crosslinking of FcεRI substantially

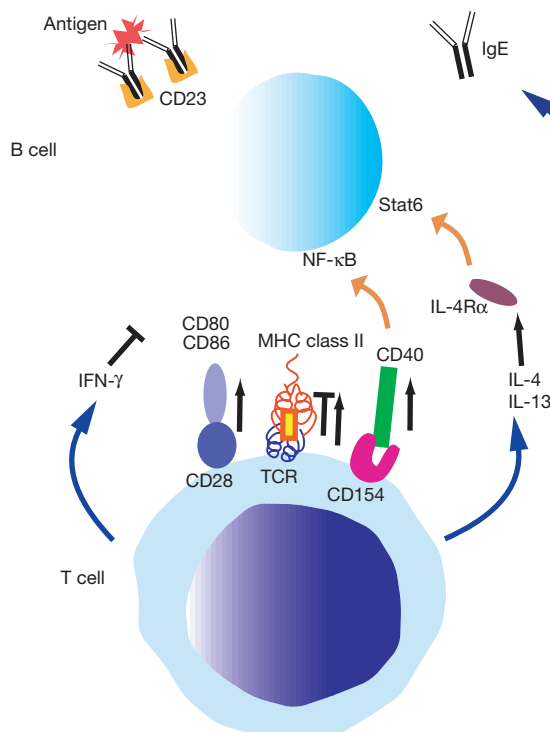


Figure 3 Molecular control of the IgE response. Although several cell types have the potential to interact with B cells to promote IgE production, T cells are probably most relevant. A stimulatory effect is indicated by $\rightarrow$; an inhibitory effect by $\dashv$. Antigen presented through MHC class II molecules to the T-cell receptor (TCR) can be either stimulatory or inhibitory depending on the nature of the antigen and other factors. CD86–CD28 (and perhaps CD80–CD28) and CD40–CD154 interactions promote IgE production

through direct effects on T cells and B cells, respectively. Although many cytokines affect IgE production, IFN- γ is probably the most important negative regulator while IL-4 and IL-13, both ligands for IL-4R α , are the most potent inducers. Although many transcription factors are probably involved, Stat-6, through IL-4R α , and NF- κ B, through CD40, directly promote IgE production by initiating epsilon transcript synthesis and class switching. The effects of CD23 are mixed, as described in Box 1

upregulates its own expression, indicating a mechanism for augmenting the biological effects of IgE where antigen is present. However, baseline IgE levels and the ability to mount a robust IgE response after immunization are not affected in the complete absence of FcεRI (J.-P. Kinet, personal communication). But as emphasized elsewhere in this supplement, care should be taken when attempting to extrapolate these experimental data to human disease.

CD23. The second major IgE receptor was identified as a binding activity present on cultured human B cells and B-cell lines. Binding studies revealed this receptor to have a 100–1,000-fold lower affinity for IgE ($K_a = 10^6$ – 10^7 M⁻¹) than the previously identified FcεRI on mast cells and basophils; consequently, the receptor was termed the low-affinity IgE receptor or FcεRII. Later studies confirmed its identity with an antigen associated with B-cell differentiation, CD23. Aside from binding IgE, CD23 shares little in common with FcεRI and does not participate directly in type I hypersensitivity reactions. Although controversial, CD23 seems to serve principally as a negative regulator of IgE synthesis but may augment IgE production under some conditions. A model for the complex functions of CD23 is described in Box 1.

Other IgE receptors and binding factors. An unusual family of T-cell-derived IgE binding factors has been described that may significantly influence the IgE response⁶⁰. The family is divided into either IgE-potentiating or IgE-suppressive factors based on their ability to augment or inhibit IgE responses, respectively. Although derived from the same gene, different modifications seem to confer the functional differences. These modifications are determined by two additional T-cell products—glycosylation inhibitory factor (GIF) and glycosylation enhancing factor (GEF). Administration of GIF to allergen-challenged mice suppresses IgE responses⁶¹, but the regulatory effects of GIF and GEF in human atopic disease,

particularly in comparison with other IgE-regulating factors, are currently unknown. Consequently, the importance of IgE-potentiating and IgE-suppressive factors remain to be established.

Although the preceding discussion has focused entirely on the negative and positive factors affecting IgE production, equally important factors determine elimination of IgE, but these are far less well understood. Human IgE binds to additional immunoglobulin receptors, including FcγRII and FcγRIII, which may mediate phagocytosis (and degradation) of IgE complexed with antigen⁶². Although this route may aid in the elimination of antigen–antibody complexes, it probably does not participate in the maintenance of basal serum IgE levels, which consist primarily of uncomplexed antibody.

Both extravascular and intravascular catabolic pathways have been identified that account for loss or degradation of serum IgE^{63,64}, although the nature of these mechanisms remain undefined. However, a recent analysis of IgE synthesis and elimination in the parasitized gut provides significant insight⁶⁵. Most IgE synthesis in response to the helminth *Trichinella spiralis* occurs within the wall of the rodent gut by terminally differentiated B cells called plasma cells. Antibody synthesized in this location is highly compartmentalized, with the vast majority transported into the lumen of the gut, probably as a result of an IL-4-inducible mechanism⁶⁶ (Fig. 4). A similar mechanism may operate in atopic syndromes: IgE is consistently found in the airways of atopic patients^{67,68}, prompting its classification as a secreted immunoglobulin⁶⁹. Intriguingly, IgE does not bind the poly-Ig transporter required for luminal export of the only other secreted antibody, IgA⁶⁷, indicating that the IgE transport mechanism involves distinct receptors or binding factors. IgE and IgA also share a suspiciously short serum half-life⁷⁰. Although much remains to be learned, the data strongly support IgE as a secreted

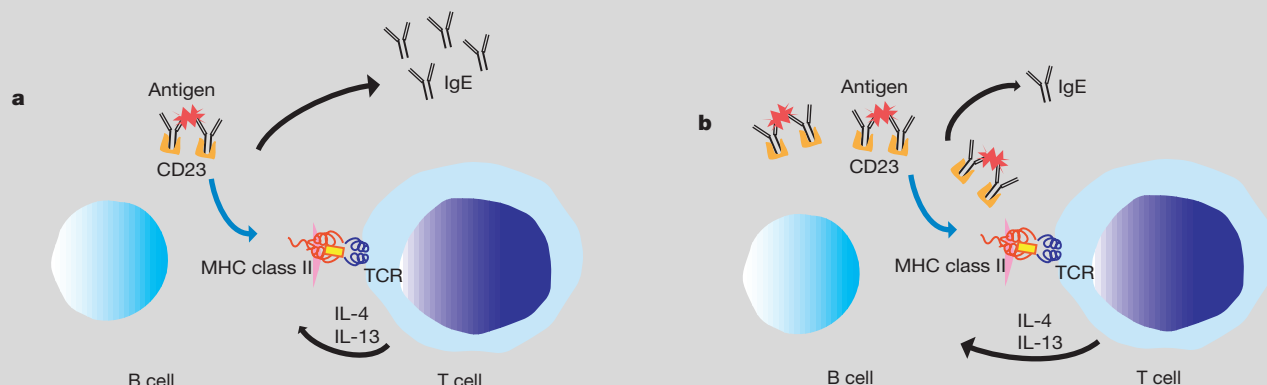
Box 1

The effects of CD23

The complex biology of CD23 hinders attempts at defining its physiological relevance, which remains speculative. Depending on the immune context, CD23 may exert opposite, but not necessarily mutually exclusive, effects on the serum IgE level. Passively administered antigen–IgE complexes augment subsequent antibody responses when administered to mice, including that for IgE, through binding to CD23 (refs 78–80). Presumably, antigen captured in this way is processed efficiently by B cells and presented to T cells for subsequent immune amplification.

However, mice entirely deficient in CD23 or with only low-level expression show increased serum IgE levels^{81,82}, particularly when antigen-specific IgE is measured⁸³. These data represent the strongest

evidence for a negative regulatory effect for CD23. The effect seems to become significant only at very high serum IgE titres, which may explain why in some studies, perhaps with less robust allergic challenges, no effect was observed^{79,84}. A model for the combined effects of CD23 is presented in the figure below. Because CD23 serves as both a positive and a negative regulator of IgE synthesis, an elegant system potentially exists for limiting IgE production and deleterious immediate hypersensitivity responses. Importantly, the distribution of CD23 differs considerably between humans and mice, and additional data are required to verify the relevance of this model in allergic disease⁸⁵.



Box 1 Figure Regulation of IgE by CD23. Based on many studies, a two-part mechanism for the role of CD23 is proposed. **a**, At an intermediate phase in allergic immune responses, when IgE levels are sufficiently high to begin binding significantly to CD23, antigen may be captured by B cells and presented to T cells, effectively augmenting IL-4/

IL-13 production and the IgE response. **b**, At later stages, however, the increased IL-4 also facilitates expression of CD23. Combined with excess IgE and antigen, CD23 becomes extensively crosslinked, providing an inhibitory signal that eventually overrides the positive effects of antigen presentation⁸⁶.

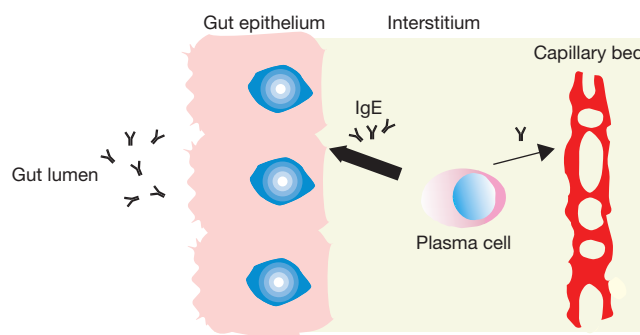


Figure 4 Elimination of IgE by extracellular transport. The majority of IgE synthesized in response to intestinal parasites occurs in the interstitium of the gut wall by terminally differentiated B cells called plasma cells. Most antibody is exported, through a poorly characterized transport system, to the lumen of the gut where presumably it participates

in extracellular host defence. Only a small fraction of the total immunoglobulin pool is delivered to the blood through the capillary bed. IgE is also detected in the airways of patients with asthma, indicating a more generalized mechanism present at multiple mucosal sites.

immunoglobulin and that, rather than undergoing elimination through catabolism, most IgE is simply secreted from the body at mucosal sites through a specific transport mechanism controlled by IL-4.

Conclusions and future directions

Although the advent of animal models of allergic disease and sophisticated molecular and immunologic reagents have provided a wealth of new information on induction and regulation of the IgE response, we are far from having a complete understanding of this important topic. In addition to gaps in existing knowledge, for example regarding mechanisms of antibody elimination, the available data present a number of intellectual and practical hurdles. Perhaps most daunting, but also most exciting, is applying the extensive knowledge gained through use of animal models to the human condition. Already the data indicate several new therapeutic avenues for amelioration of allergic disease, including strategies that target IL-4, IL-13 and their receptors.

An equally important challenge will be resolving the discrepancies that inevitably arise when results from experimental models are compared to human diseases. For example, a central paradox of allergy has been the existence of both atopic (extrinsic) and non-atopic (intrinsic) syndromes, the latter representing patients with atopic disease but no serum IgE or skin reactivity to common allergens. Several explanations have been proposed to resolve this discrepancy, the simplest being that the 'correct' antigen has simply yet to be found in non-atopic patients. Data from animal models, however, indicate a different explanation. Recently, the mechanism underlying the experimental asthma phenotype has been found to be largely antibody independent^{34,71}, although IL-4 and IL-13 remain critical components^{72,73}. Because these cytokines are also essential for the IgE response, the data suggest that IgE may be a marker of asthma-like disease only under some conditions. Another experimental atopic disease, anaphylaxis, may occur in the total absence of IgE^{74,75}. These data stand in contrast to the previously demonstrated role of IgE in both experimental anaphylaxis and asthma^{76,77}.

How seriously do these findings challenge our long-held notions of the role of IgE in disease? Again, extrapolating from rodent systems to humans is potentially misleading. However, the data suggest a more complex pathogenic landscape than previously suspected, implying that the role of IgE in allergy has yet to be fully explored. The finding that IgE may be produced mucosally and exported to the lumen under the control of IL-4 indicates that serum and skin-based assays may sometimes be inadequate for investigating the role of IgE in atopic disease. Indeed, because most IgE may be secreted intraluminally, many non-atopic asthmatics might be found to be atopic if the IgE content of mucosal fluids were

determined. Our evolving concepts of IgE regulation will remain important tools as we begin reassessing the role of IgE in health and disease. □

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Signalling through the high-affinity IgE receptor FcεRI

Helen Turner & Jean-Pierre Kinet

The FcεRI complex forms a high-affinity cell-surface receptor for the Fc region of antigen-specific immunoglobulin E (IgE) molecules. FcεRI is multimeric and is a member of a family of related antigen/Fc receptors which have conserved structural features and similar roles in initiating intracellular signalling cascades. In humans, FcεRI controls the activation of mast cells and basophils, and participates in IgE-mediated antigen presentation. Multivalent antigens bind and crosslink IgE molecules held at the cell surface by FcεRI. Receptor aggregation induces multiple signalling pathways that control diverse effector responses. These include the secretion of allergic mediators and induction of cytokine gene transcription, resulting in secretion of molecules such as interleukin-4, interleukin-6, tumour-necrosis factor-α and granulocyte-macrophage colony-stimulating factor. FcεRI is therefore central to the induction and maintenance of an allergic response and may confer physiological protection in parasitic infections.

Three distinct protein species compose the FcεRI complex¹. The α-chain binds the Fc portion of IgE* with high affinity, the β-chain has four transmembrane domains between amino- and carboxy-terminal cytoplasmic tails, and a homodimer of two disulphide-linked γ-chains completes the tetrameric structure. In humans, the tetrameric structure is not obligatory—an alternate form is present, comprising an αγ₂ trimer. On the basis of several similarities in structural features and signalling targets, FcεRI has been placed in an *antigen* receptor superfamily with the B- and T-cell antigen receptors (BCR, TCR) and several immunoglobulin-γ (IgG) Fc receptor isotypes (Fig. 1)^{1–3}. First, the α-chain contains two immunoglobulin-type domains, and similar domains are found in the ligand-binding subunits of other superfamily members. Second, the β- and γ-subunits of FcεRI contain conserved immunoreceptor tyrosine-based activation motifs (ITAMs) in their cytoplasmic tails^{3,4}. These motifs are phosphoacceptors, through which the receptor subunits interact with signalling proteins. Finally, there is considerable homology between the signalling pathways initiated by FcεRI and other antigen receptors.

FcεRI binding of IgE

Signalling through the FcεRI is initiated by crosslinking of multivalent antigen bound to IgE; it is a prerequisite that IgE monomers have previously bound to the receptor through their Fc regions. Studies of the interaction between IgE and FcεRI have shown that the stoichiometry of the interaction is 1:1 (ref. 5) and that the interaction is high affinity ($K_d = 10^{-9}$ – 10^{-10} M). In addition, mutational analyses indicate that the third Ig constant domain (Cε3) of IgE may form the contact point between antibody and ligand^{6,7}, and that the α-subunit is highly N-glycosylated but that glycosylation is not necessary for IgE binding^{8,9}. After much effort, the crystal structure of the FcεRIα chain has now been determined, providing some insight into the nature of the IgE interaction with its Fc receptor¹⁰.

Crystallization reveals that the two Ig domains (D1 and D2) of FcεRIα are positioned at an acute angle to one another, forming a convex surface at the top of the molecule and a marked cleft enclosed by the two globular immunoglobulin domains (Fig. 2a). The relative orientation of these two domains differs between the various structures where tandem immunoglobulin domains are present and is determined by the composition of the interface region. Amino acids in the cleft are directed towards the membrane and will not interact with IgE. Residues forming the top surface of

the protein have previously been implicated in binding to the IgE Fc portion. Here, four grouped tryptophan residues (contributed by D1, D2 and the interface region) form a hydrophobic patch that is a putative contact point for the binding of IgE Fc.

The proposed relationship between IgE and FcεRIα molecules is shown schematically in Fig. 2b. The IgE Fc region is homodimeric. Although two possible surfaces for interaction between FcεRIα and IgE Cε3 exist, the interaction stoichiometry is 1:1 (ref. 5). There are several possible reasons why two FcεRIα molecules are never bound to the two antibody immunoglobulin domains. First, the binding of one FcεRIα to IgE may partly occlude the other immunoglobulin domain and prevent, sterically, the interaction of a second receptor. Second, a conformational change may follow FcεRIα and IgE interaction, such that the Fc structure alters to prevent binding to another receptor.

IgE crosslinking by multivalent antigen

The IgE/FcεRIα interaction is followed by the antigenic crosslinking of receptors in the plane of the plasma membrane. FcεRIα chains do not aggregate in the absence of antigen, possibly as a result of the extensive glycosylation of the front and back of the molecule. This glycosylation does not extend to the top surface, where the IgE interaction is proposed to take place. *In vitro*, deglycosylated FcεRIα aggregates spontaneously without antigen. The glycosylation of potential α-chain interaction surfaces prevents premature aggregation and permits interaction with the synthetic machinery⁹, whereas binding of multivalent antigen overcomes the intrinsic resistance of α-chains to interaction.

FcεRIα is not a conventional signal transducing molecule. It has a short (17 amino acids) cytoplasmic tail that has never been found to interact with a signalling target. In fact, deletion of the entire FcεRIα cytoplasmic domain does not compromise FcεRI signalling¹¹. However, information concerning productive ligation of α-chains is passed to the β- and γ-chains (the signalling subunits) of the receptor, and an aggregation-induced change in FcεRIα must be sensed by the β- and γ-subunits. The crystal structure of FcεRIα now provides an enticing possibility that residues in the cleft formed by the opposition of D1 and D2 could interact with the extracellular portion of the β- or γ-subunit and transmit information regarding aggregation.

Early FcεRI signalling events

Antigenic crosslinking of the FcεRI initiates a chain of phosphate transfer events within the receptor microenvironment. The system of information transfer relies on the ligation-dependent recruit-

* Terms in *italic* are defined in the glossary on p. B39.

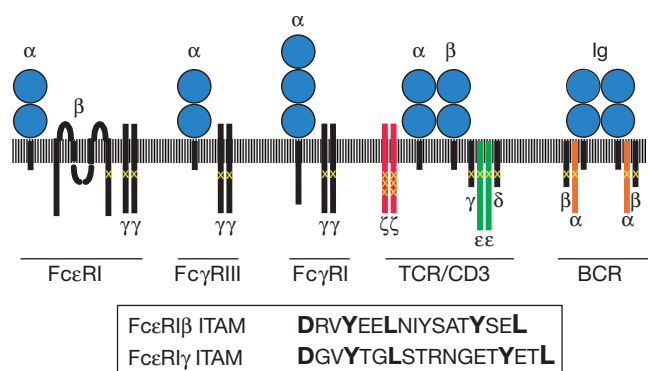


Figure 1 ITAM-containing immunoreceptors. The tetrameric FcεRI is shown in the context of other members of the immunoreceptor superfamily. Blue circles represent immunoglobulin domains. ITAM motifs are shown as yellow crosses. TCR, T-cell antigen receptor; BCR, B-cell antigen receptor; Ig, immunoglobulin; ITAM, immunoreceptor tyrosine-based activation motif. Inset: sequences of the ITAM motifs contained within the β- and γ-chains of FcεRI. Canonical residues are shown in bold type.

ment of assembled signalling cassettes into the receptor context, providing an organizational parallel with the early events that occur after ligation of other antigen receptors. Phosphate transfer assists in this recruitment, and in the subsequent activation of recruited signalling proteins.

The β- and γ-chains of the FcεRI complex contain ITAMs, which are a conserved feature of antigen and some Fc receptors^{1–4,12}. The ITAM consensus sequence is D/E-XX-YXXL-X_{7–11}-YXXL-L/I, where the tyrosine residues are phosphoacceptor sites for the action of receptor-associated protein tyrosine kinases (PTKs)⁴. Phospho-ITAMs provide a docking site for cytoplasmic proteins that contain the Src-homology-2 (SH2) domain and hence link receptor and signal transduction cascades, as the SH2 domain has high affinity for phosphorylated tyrosine residues¹³. In the context of FcεRI, the β- and γ-chain ITAMs have slightly different structures (Fig. 1). The β-chain ITAM is notable owing to its differences from the consensus ITAM sequence; the presence of a third tyrosine between the canonical tyrosine residues; and the short length of its spacer region. The β- and γ-chain ITAMs also have distinct functions. There are two species of FcεRI-associated PTK—the *src* family kinase Lyn and p72Syk kinase. The former is found associated with FcεRIβ, whereas the latter is able to bind both FcεRIβ and FcεRIγ but has higher affinity for interaction with FcεRIγ.

Several studies have contributed to a model for the events that occur immediately after FcεRI aggregation^{1,14}. The salient features of this model are as follows. First, the β- and γ-chains act cooperatively. Both *in vitro* and genetic reconstitution studies illustrate this point. Reconstitution of FcεRI-deficient *mast-cell* lines showed that mutation of the two canonical tyrosines in the β-chain ITAM abolished activation-dependent phosphorylation of both the β- and the γ-chain ITAMs¹⁵, which indicates that the phosphorylation of the former has bearing on the status of the latter. Moreover, whereas Lyn^{−/−} mast cells exhibit no β- or γ-chain phosphorylation¹⁶, Syk^{−/−} cells have intact β- and γ-chain phosphorylation but still lack downstream signalling events^{17,18}. Second, Lyn binds to FcεRIβ under resting conditions. An obvious candidate for the mediation of this interaction is the Lyn SH2 domain, as FcεRIβ is slightly tyrosine-phosphorylated under resting conditions. However, we have shown that the Lyn SH2 domain is not required for FcεRI phosphorylation (S. Lin and J.P.K., unpublished data), and others have demonstrated that the ‘unique’ (SH4-containing) domain of Lyn interacts with FcεRIβ¹⁹. In addition, Lyn is likely

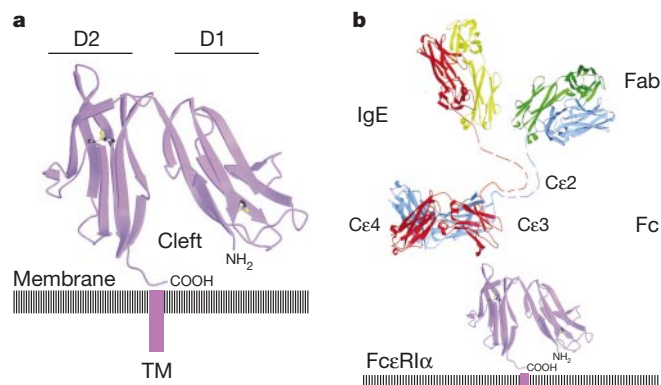


Figure 2 Structural relationship of FcεRIα and its ligand, IgE. **a**, Schematic structure of the FcεRIα chain. The opposing immunoglobulin domains, D1 and D2, form a marked cleft lined by residues that face the plasma membrane. TM, transmembrane domain. **b**, Interaction of IgE and FcεRIα. Contact residues have been localized to the top of D2 in FcεRIα, and the Cε2:Cε3 junction in the Fc region of IgE. Fab, antibody binding fragment; Fc, constant region.

to rest passively in the receptor context owing to its myristoylation, which constitutively targets the enzyme to the membrane. Third, active Lyn phosphorylates the β- and γ-chain ITAMs. Upon receptor aggregation, Lyn is activated and its catalytic activity becomes directed towards the β- and γ-chain ITAMs. Syk is then recruited to the receptor γ-chain through one of the tandem SH2 domains in the kinase¹⁸. An important feature is that Lyn may transphosphorylate ITAM in other receptor complexes²⁰. Fourth, Syk binding to FcεRIγ leads to Lyn-dependent tyrosine phosphorylation and activation of the kinase. This step enables the productive interaction of active Syk with its many targets²¹.

One step that remains unclear in the initiation of the early events in FcεRI signalling is how FcεRIα aggregation causes transition of Lyn from an inactive to an active state. Moreover, analyses of the CD45 phosphatase and Src kinases indicate that there may be subtle subdivisions in the activation status of Lyn. In its inactive/closed state, Lyn is phosphorylated on a C-terminal regulatory tyrosine by the Src kinase Csk²². This phosphotyrosine provides a docking site for the Lyn SH2 domain, which binds and occludes the activation domain of Lyn²³. Moreover, by analogy with studies on Hck, there is also an intramolecular interaction of the Lyn SH3 domain that must be displaced before the kinase is fully activated. The first of these intramolecular interactions is thought to be released by dephosphorylation of the inhibitory tyrosine residue by CD45. The displacement of the intramolecular SH3 interaction is not well understood, but it seems likely that some aggregation-induced change in a receptor subunit or associated molecule provides a higher affinity SH3 ligand that successfully competes for the Lyn SH3 domain. The SH3 domain is directed towards proline-rich regions¹³, and such a ligand has not yet been identified in the receptor context. Lyn then moves into an inactive/open conformation, priming the kinase for activation²⁴. Autophosphorylation of open Lyn is then required for full activity of the kinase.

The mechanism by which α-chain aggregation is sensed by the signalling subunits and subsequently alters the interplay between CD45 and Lyn, Lyn and Csk, and Lyn with other possible molecules (such as SHP phosphatases) is unknown (Fig. 3a). CD45 does not, however, continue to dephosphorylate either the activatory tyrosine on Lyn or other phosphotyrosines in the receptor context. It therefore seems likely that, after aggregation, CD45 is actively excluded from this context, perhaps as a result of the partition of receptor aggregates into lipid microdomains^{24,25}.

Initiation of intracellular signalling cascades

Activation of Lyn and Syk drives intracellular signal transduction cascades that regulate cellular functions. Some basic principles that underlie the complex network of events occurring immediately proximal to the receptor can be identified. First, signalling molecules are recruited to the receptor context; second, a post-translational modification (for example, tyrosine phosphorylation) activates the catalytic activity of the signalling protein; and third, pluripotent adapter proteins have inducible or constitutively associated effector proteins that direct their activity towards a particular intracellular target.

One of the primary targets of antigen-receptor signalling is phospholipase $C\gamma 1$ (PLC $\gamma 1$), which catalyses the breakdown of membrane phospholipids to generate two second messengers—inositol-1,4,5-trisphosphate (Ins(1,4,5)P₃) and diacylglycerol (DAG). These signalling molecules are responsible for calcium release from intracellular stores and activation of various protein kinase C (PKC) isoforms, respectively. PLC $\gamma 1$ must be recruited to the membrane (the location of its substrate pool) and tyrosine-phosphorylated for activation. In T cells, this recruitment is accomplished by the linker for activation of T cells (LAT) adapter protein²⁶, a membrane anchored substrate for the Syk analogue ZAP-70 (ref. 27). Mast cells have recently been shown to contain LAT²⁸, and it is likely that T and mast cells share this LAT-dependent mechanism for PLC $\gamma 1$ membrane targeting. Once within the membrane, PLC $\gamma 1$ is exposed to activating tyrosine phosphorylation. Two types of kinase contribute to this process—Syk and either the tyrosine kinase Itk or Bruton's tyrosine kinase (Btk), both of which are members of the Tec family of non-receptor tyrosine kinases. As described above, Syk activation derives from membrane targeting followed by Lyn-dependent phosphorylation, leading to full activation. Similarly, Btk is first membrane-targeted by binding of a phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P₃) moiety to its pleckstrin-homology (PH) domain^{29,30}. It then becomes an accessible substrate for Lyn and is able to autophosphorylate, giving full activation³¹. Btk then phosphorylates and activates PLC $\gamma 1$, a process requiring the adapter Slp-76.

Btk controls PLC $\gamma 1$ tyrosine phosphorylation, and Btk recruitment/activation requires PtdIns(3,4,5)P₃ (ref. 32). In turn, this raises the question of how Fc ϵ RI directs PtdIns(3,4,5)P₃ synthesis. PtdIns(3,4,5)P₃ derives from phosphorylation of pre-existing phosphatidylinositol-4,5-bisphosphate, a reaction catalysed by phosphatidylinositol-3-OH kinase (PI(3)K)³³. PI(3)K comprises a regulatory subunit of relative molecular mass 85,000 (M_r 85K), which is tyrosine phosphorylated by receptor-associated PTKs, and a catalytic subunit (M_r 110K), which must be recruited to the membrane in order to locate its substrate pool. One model is therefore that receptor-associated PTKs phosphorylate p85, which causes activation and contributes to recruitment of p110. We have recently shown that Syk is required for PI(3)K activation in mast cells (L. Beitz and J.P.K., unpublished data), potentially placing Syk upstream of p85 tyrosine phosphorylation, or indirectly affecting p110 recruitment, or both.

Membrane proximal adapter proteins are important in early Fc ϵ RI signalling. Adapter proteins are common in antigen-receptor signalling cascades, are composed of modular protein–protein interaction domains and lack intrinsic enzymatic activity. Adapter proteins are excellent examples of the modular design of signalling proteins, which have evolved conserved structural domains that allow for diversification of function within a single polypeptide¹³.

The Grb-2 adapter comprises two SH3 (proline-directed) domains and one SH2 (phosphotyrosine-directed) domain. Grb-2 links upstream phosphotyrosine residues (substrates for receptor-associated PTKs) and effector molecules that form complexes with its SH3 domain. Grb-2 does not adapt directly to Fc ϵ RI ITAMs. Rather, indirect association is mediated either by the Shc adapter or by the LAT phosphoprotein. In mast cells, two prominent Grb-2

effector molecules are Sos and Slp-76 (ref. 34).

Grb-2 contributes to the recruitment and presumably the orientation of Sos towards its substrate, the Ras GTPase. Sos may also be membrane-targeted by its PH domain/phospholipid interaction³⁵, but it is unclear whether this assists, precedes or follows the Grb-2 interaction. Sos is a guanine nucleotide exchange factor for Ras, a membrane-resident GTPase. Sos promotes GTP loading and hence activation of Ras and its multiple downstream effector pathways, including activation of other GTPases (in fibroblasts, there is crosstalk at the level of Sos between Ras and Rac GTPase activation³⁶), PI(3)K and the Raf/MEK/ERK cascade³⁷.

Slp-76 protein is a critical effector in antigen signalling and can apparently act at several different positions in the signalling network. In T and mast cells it has been shown that one function of Slp-76 is to recruit Vav (itself a substrate for receptor-associated PTKs)³⁸. Vav is a guanine nucleotide exchange factor for the Rac GTPase³⁹, and thus through Slp-76 a further set of GTPase effector pathways are initiated (cytoskeletal rearrangements, p21-activated kinase (PAK) activity and Rho activation). However, in T cells there is also clear genetic evidence that a deficiency in Slp-76 uncouples receptor-associated PTKs from PLC $\gamma 1$ activation, placing Slp-76 upstream of both Ins(1,4,5)P₃ generation and Ras activation in the same cell type²⁷. This pathway probably operates also in mast cells.

However, new data obtained from T cells may complicate the model for mast cells. Although Grb-2 can mediate the interaction between LAT and Slp-76, it now seems that this is also accomplished by GADS, a novel haematopoietic adapter protein⁴⁰. GADS also binds Shc, and it is unclear whether Grb-2 and GADS complexes coexist in the mast cell and what their relative roles are in downstream signalling pathways. Where multiple routes to a downstream target exist, it is likely that subtle choices are made by receptors and that these may not be visible in current assay systems.

GTPase/kinase networks

As described above, Fc ϵ RI aggregation results in activation of guanine nucleotide exchange factors that promote the GTP loading, and hence increased activity, of the small GTPases Ras, Rac and Rho. This class of proteins receive and react to information on many cellular processes. In the context of the mast cell, the involvement of GTPases in transcriptional activation and secretion of allergic mediators has been described.

It is well established that each GTPase has available a wide range of effector molecules³⁷, each of which in turn regulate complex networks of signalling pathways such as mitogen-activated protein (MAP) kinase cascades. The terminal targets of these pathways include transcription factors. For example, in the mast cell, the Ras GTPase initiates a classical Ras/Raf-1/MEK/ERK signalling cascade that targets the Elk-1 transcription factor, which is involved in immediate early gene regulation. Rac-1, on the other hand, uses an unknown effector pathway to regulate the subcellular location of the cytokine gene transcription factor, NFAT^{41,42}. Both Ras and Rac-1 are involved in regulation of the AP-1 class of transcription factors, which independently regulate some sites and act in concert with NFAT.

Some GTPase pathways do not have nuclear targets, as two studies using mast cells have shown. First, GTP γ S, which activates all classes of GTPase, can stimulate secretion of allergic mediators in mast cells. Because this is an exocytotic process, some groups have addressed the question of whether the Rab class of GTPase, which is involved in vesicle trafficking, may be important in regulation. Indeed, dominant inhibitory forms of Rab3d do block antigen-driven exocytosis⁴³. Second, profound morphological change is associated with Fc ϵ RI aggregation on mast cells, including the formation of membrane ruffles and filopodia. The role of Ras, Rac and Rho GTPases in regulating such changes is well established in fibroblasts⁴⁴ and similar mechanisms exist in the mast cell.

Elevation of intracellular free calcium

FcεRI aggregation causes a biphasic modulation in intracellular free calcium ($[Ca^{2+}]_i$). First, $Ins(1,4,5)P_3$ generated by PLC γ 1 binds and opens the $Ins(1,4,5)P_3$ receptor calcium channels on the surface of intracellular calcium stores (primarily the endoplasmic reticulum (ER)), which are then depleted of calcium. The cell is now presented with a problem: the finite amount of stored calcium is soon exhausted and cannot deliver sustained calcium signals. Moreover, stored calcium needs to be replenished. FcεRI-effector functions rely on sustained elevation in $[Ca^{2+}]_i$. For example, transcriptional activation of cytokine genes in mast

cells, such as the gene for interleukin (IL)-4, requires sustained increases in cytoplasmic calcium^{41,45}. Here, calcium signals are required for the dephosphorylation and nuclear import of the transcription factor NFAT, as NFAT is dephosphorylated by the calcium-dependent phosphatase calcineurin. If calcium is removed, rephosphorylation by a nuclear resident kinase occurs and NFAT is exported to the cytosol where it no longer contacts its target genes⁴⁶.

It is also apparent that sustained calcium signals act in synergy with those provided by cytoplasmic kinases to induce secretion in mast cells. Generalized activation of PKC and, indirectly, other

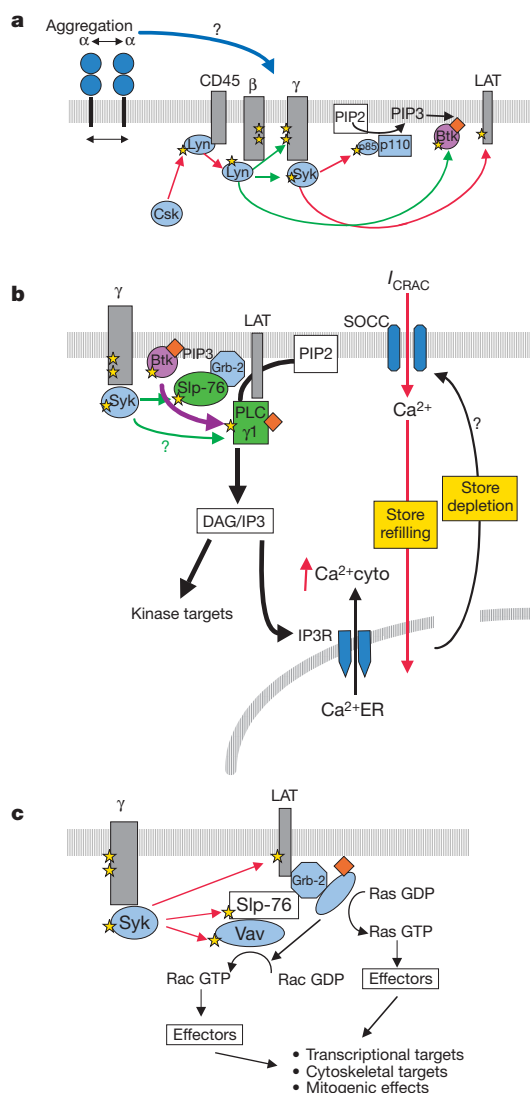


Figure 3 FcεRI activation induces complex networks of signalling events. **a**, Early events in FcεRI signalling. FcεRIα chains are aggregated by crosslinking of bound IgE by multivalent antigen. Information concerning aggregation is passed, by an unknown mechanism, to the β- and γ-chain signalling subunits. Lyn activation status is defined by the opposing actions of the CD45 phosphatase and the Csk kinase. Activated Lyn phosphorylates the β- and γ-chain ITAMs, the latter is then able to recruit Syk, which is in turn activated by Lyn. Targets of Lyn and Syk include the activation of PI(3)K to produce $PtdIns(3,4,5)P_3$ (PIP3), phosphorylation of the BTK and phosphorylation of the membrane-localized adapter protein LAT. **b**, FcεRI control of $[Ca^{2+}]_i$. BTK, which is localized at the membrane by binding to PIP3, contributes to activation of PLCγ1, which in turn has been brought to the membrane by the phosphorylated LAT adapter. PLCγ1 acts on membrane inositol phospholipids to generate $Ins(1,4,5)P_3$ (IP3) and DAG. The intracellular targets of the latter include PKC isoforms, whereas IP3 binds to the IP3 receptors (IP3R) on the

surface of the ER calcium stores, leading to store depletion and elevation of cytoplasmic calcium levels. Depletion of ER calcium stores is coupled to opening of plasma membrane channels for calcium influx; the calcium release activated current (I_{CRAC}) is mediated by these store-operated calcium channels (SOCC), and is responsible for sustained elevations in cytosolic calcium and refilling of ER stores. **c**, FcεRI-regulated adapter molecules contribute to activation of GTPase/kinase cascades. Syk targets include the LAT adapter molecule, which anchors Grb-2 and Slp-76 adapters. These in turn contribute to the recruitment/localization of guanine nucleotide exchange factors for Ras family GTPases. GTP loading of GTPases initiates a wide range of effector responses. These include the induction of serial kinase cascades such as the Ras/Raf-1/MEK/ERK cascade, where the terminal targets are transcription factors, and the rearrangement of the actin cytoskeleton to produce changes in cell morphology. Diamonds represent PH domains/phospholipid interaction; stars represent phosphorylations.

signalling pathways using phorbol esters induces sub-maximal secretion. Combination with sustained calcium signals from ionophore treatment causes a synergistic increase in secretion, although ionophore alone is not a strong secretory stimulus. If external calcium is removed (that is, if influx ceases), secretion is halted abruptly. In the presence of a calcium chelator such as EGTA, the Fc ϵ RI itself cannot induce significant secretion. The precise targets for these calcium/PKC signals within the secretory machinery in mast cells are not known.

The second phase of calcium signalling induced by Fc ϵ RI satisfies the requirement for sustained calcium signals. Store depletion causes the influx of calcium from the surrounding environment (Fig. 3b). It is not yet clear how store depletion is coupled to this influx, but it is thought that a class of 'store-operated' calcium channels (SOCC)⁴⁷ mediates influx. These channels are defined by their opening in response to any store-depletion stimulus, and they are thought to be distinct from known channel families such as the Trp channels or voltage-operated calcium channels. In mast cells, where voltage-operated or ligand-gated calcium channels are not expressed, the sole entry mechanism for calcium is thought to be through SOCCs. More specifically, the calcium release activated current, I_{CRAC} , has been defined, in electrophysiological terms, as the primary mechanism for calcium entry and store refilling in mast cells^{48,49}. I_{CRAC} is a highly calcium selective cation current but the channel proteins underlying the I_{CRAC} current and their regulatory mechanisms are unknown. This remains a major question in immunological research, as most lymphoid and mast cells are reliant on these channels for the sustained calcium influx needed to drive cellular activation.

Turning the receptor off

The potent mast-cell effector function initiated by Fc ϵ RI does not last indefinitely. Activation persists only as long as the receptor is engaged and phosphorylated⁵⁰. A unique feature of Fc ϵ RI among antigen receptors is that its engagement with multivalent antigen can be reversed rapidly by the addition of monovalent hapten⁵⁰. Signalling is terminated completely by receptor disengagement from multivalent antigen, indicating that rapid response mechanisms are in place to stop the induction of signalling pathways in the absence of continued stimulation. Although it is unlikely that receptor disengagement by competing monovalent antigen would occur *in vivo*, it is worthwhile examining the underlying mechanisms for receptor deactivation.

The off-rate of multivalent antigen is likely to be slow and cannot be relied upon as a method of signal termination, in contrast with other antigen receptors where the on- and off-rates of short peptide ligands are relatively fast. In addition, rapid receptor internalization that follows crosslinking may not be an effective signal terminator, as competent signalling complexes may still be present. Phosphorylation of phosphoacceptors in both receptor subunits and intracellular targets initiates and maintains signalling. Therefore, as a receptor-level termination mechanism, dephosphorylation by specific phosphatases seems likely.

The β -chain ITAM binds, *in vitro*, to the protein tyrosine phosphatases SHP1 and SHP2, and the inositol phospholipid-directed phosphatase SHIP also associates with Fc ϵ RI^{51,52}. However, the SHP2 interaction has been regarded as irrelevant owing to its low affinity as assayed by surface plasmon resonance⁵³. Our recent data suggest that SHP1 is most important in termination of Fc ϵ RI signals (D. Lin and J.P.K., unpublished data). By analogy with the BCR, SHIP is likely to be important in terminating signalling by lipid second messengers. Open questions still concern the preference or specificity of the phosphatases deactivating Fc ϵ RI, and the nature of their own activation/deactivation cycles.

Tuning the receptor

Studies using chimaeric receptors have shown that the Fc ϵ RI γ /Syk

complex is competent to drive cell activation in the absence of a β -chain ITAM, so prompting the question 'What is the role of Fc ϵ RI β ?'. We have observed that, *in vitro* and *in vivo*, Fc ϵ RI β acts as a signal amplifier^{54,55}: Fc ϵ RI β does not have autonomous cell-activation properties but does produce a five- to sevenfold amplification in the intensity of signals from Fc ϵ RI γ (as measured by Syk activation and calcium mobilization). The mechanism of amplification involves enhanced Lyn recruitment and increased Lyn phosphorylation of Fc ϵ RI γ . *In vivo*, we have shown exhaustively that Fc ϵ RI β is an amplifier of responses using humanized Fc ϵ RI α transgenic mice expressing either $\alpha\gamma_2$ or $\alpha\beta\gamma_2$ complexes at the mast-cell surface. The amplifying effect of Fc ϵ RI β is observed *in vivo* at the level of receptor phosphorylation, calcium mobilization, mediator secretion and IL-6 generation. Even *in vivo* anaphylaxis was enhanced threefold in $\alpha\beta\gamma_2$ complexes compared with $\alpha\gamma_2$ complexes.

We have recently explored whether enhancement of signalling by surface-resident receptors is the only mechanism by which Fc ϵ RI β exerts its amplifying effect. In fact, there seems to be a second role for Fc ϵ RI β in that it associates with $\alpha\gamma_2$ complexes early in biosynthesis and promotes the transport of intracellular Fc ϵ RI α chains from ER to Golgi, the latter being where the α -chain is terminally glycosylated. Detergent studies revealed that the β -chain stabilizes the Fc ϵ RI complex, leading us to the final observation that the β -chain positively affects Fc ϵ RI surface expression; the presence of β -chains markedly increases the number of surface α -chains (fully assembled receptors) at the surface of transfected cells (E. Donnadieu and J.P.K., unpublished data).

The presence of an intrinsic molecular amplifier is so far unique to the Fc ϵ RI among antigen receptors. It is unclear whether certain subunits of other multimeric receptors can modify the intensity of signalling. However, in the case of Fc ϵ RI, small changes in β -chain function could profoundly affect signalling and the resulting effector functions controlled by Fc ϵ RI. It is therefore intriguing to note that genetic studies have revealed that a locus in the long arm (q) of chromosome 11 at bands 12–13 (11q12–13), which contains the Fc ϵ RI β gene, is linked with the allergic disorder *atopy*^{56,57}.

Further analysis has revealed that several *polymorphisms* of Fc ϵ RI β associate with allergic phenotypes in various populations. These include the substitutions I181L and V183L, usually observed in tandem, and E237G^{58,59}. We investigated *in vitro* whether mutant Fc ϵ RI β containing the former mutations, singly or doubly, or the latter single substitution, functioned differently than wild-type Fc ϵ RI β . We assayed the signal-amplifier effect on calcium mobilization and the trafficking-based effect of the β -chain on Fc ϵ RI surface expression. None of the β -chain polymorphisms tested enhanced or diminished Fc ϵ RI β function. However, it is appropriate to note that polymorphisms in Fc ϵ RI β may, in fact, be in *linkage disequilibrium* with another genetic factor that is phenotypically associated with atopy.

There is one further level at which Fc ϵ RI regulates its own function. Fc ϵ RI levels at the surface of mast cells and *basophils* are modulated by the serum IgE concentration; receptor occupancy seems to increase receptor levels at the cell surface. This is an important mechanism as there is a concomitant increase in the severity of effector responses⁶⁰. Ligand-mediated Fc ϵ RI upregulation is seen in rodent and human, and is not dependent on Fc ϵ RI structure ($\alpha\beta\gamma_2$ compared with $\alpha\gamma_2$). The upregulation is biphasic: a cyclohexamide-insensitive phase precedes a sustained phase that is highly dependent on protein synthesis. IgE-mediated amplification can be modelled as follows. First, IgE binding stabilizes receptor complexes at the surface and protects them from degradation, the result being surface Fc ϵ RI accumulation from the intracellular pool without the need for *de novo* synthesis. Later, when all intracellular Fc ϵ RI is at the surface, continued upregulation could be maintained only by the synthesis of new complexes from pre-existing transcripts.

The modular nature of FcεRI: implications for animal models

In the field of signal transduction, there has been a general progression from the study of *in vitro* interactions between signalling molecules in cell lines towards the assignment of relevance to such interactions *in vivo*, followed by study of their possible roles in pathology. Because allergy and atopy are diseases of the immune system with signal transduction at their core, it is tempting to extend these *in vivo* analyses to the study of mast-cell signalling pathways. However, there are major differences between FcεRI complexes in mouse and human systems that should be noted.

Rodent FcεRI has an obligatory tetrameric αβγ₂ structure. In humans, both αγ₂ and αβγ₂ complexes are observed. Rodent FcεRI complexes are confined to the surface of mast cells and basophils. In humans, however, it is now recognized that there is a far wider distribution of FcεRI. On the mast cell and basophil surface there is a mixture of αβγ₂ and αγ₂ complexes, whereas *monocytes*, *Langerhans cells*, *eosinophils* and *dendritic cells* express surface αγ₂ (ref. 1). Finally, in rodent but not human there is crosstalk between IgE- and IgG-mediated cellular activation. In the rodent, the IgE Fc region can bind to two classes of low-affinity FcγRII, that is, activatory and inhibitory isotypes⁶¹. Both of these are expressed on mast cells and so there is a route by which IgE or IgG immune complexes may regulate mast-cell function independently of FcεRI.

The first question to address is why the rodent FcεRI reaches the cell surface only as an assembled tetramer. Transfection experiments showed that rodent α-chain contains an ER retention signal that is masked by co-transfection of, and assembly with, βγ₂; this allows export to the cell surface. In humans, however, there is αγ₂ at the cell surface with no β-chain, indicating that the γ-chain alone can balance the human ER retention signal. Why is this not sufficient in rodent? Our data indicate that an additional ER retention signal is placed in the rodent extracellular domain of the α-chain⁶². How this would be deactivated by FcεRIβ to allow surface expression is not clear, although it is now possible to infer from the crystal structure of the α-chain that the D1/D2 cleft could interact with the extracellular portion of FcεRIβ.

Second, in view of the model presented above, where FcεRIβ and Lyn are required for Syk recruitment and activation, we have to ask how the αγ₂ complex accomplishes competent signalling. The answer to this question may lie in the fact that recruitment to FcεRIβ is not the only mechanism by which Lyn is membrane-targeted. Lyn is myristoylated in its N-terminal 'unique' domain, allowing it to interact with the membrane. In αγ₂ complexes, a population of Lyn would in fact be present, anchored passively in the receptor context by myristoylation. Here, Lyn will contribute to FcεRIγ/Syk activation, but less efficiently than where specific recruitment and orientation by FcεRIβ occurs.

We should also consider that there is a wider cell distribution of FcεRI in humans than rodents. In humans, FcεRI was detected on normal dendritic and Langerhans cells, as well as the eosinophils of hypereosinophilic patients and monocytes of atopics. In the latter cell types the αγ₂ receptor has been shown to have a significant role in IgE-mediated antigen presentation^{63,64}. The level of FcεRI expression on these cell types is very low, presumably due to the lack of FcεRIβ to assist in trafficking. Because αγ₂ complexes are less competent at signalling than the αβγ₂ on mast cells and basophils (they lack the β signal amplifier), the function of antigen presentation by the antigen-presenting cells (APCs) may be accomplished with only moderate induction of intracellular signalling pathways. Antigen presentation by αγ₂ complexes on APCs does lead to some intracellular signalling⁶³, which is possibly involved in delivering co-stimulatory signals to T cells. Thus the antigen-presenting function of FcεRI provides a direct link between IgE-mediated responses and T cells, and this may be critical to the long-term maintenance of an IgE-driven immune response.

IgE-mediated antigen presentation occurs in humans but not

rodents. Moreover, there are other differences between the two systems that lead us to question the validity of rodent models for human allergy. In rodent, multimeric IgE immune complexes can interact with activatory and inhibitory Fcγ receptors (FcγR) on the surface of mast (and other) cells. Obviously, IgG immune complexes can also interact with these FcγR. In humans, there is no crosstalk between IgE- and IgG-mediated responses, so IgE, monomeric or in immune complex, cannot interact with FcγR. This has a particular bearing on *in vivo* experiments, which challenge immunized rodents with high levels of antigen (often delivered intravenously). These antigen doses will form IgE or IgG immune complexes, which will be directed towards FcγR, and the activatory consequence of this will be an immune-complex-based disease state, or Arthus reaction. Small amounts of antigen could in theory drive a monomeric IgE/FcεRI reaction in mice, but in this delivery system it would tend to form immune complexes in the blood and be destroyed as such in the spleen before development of a true allergic reaction. Conversely, in humans, the tiny amounts of allergen that are delivered locally (to the skin and lungs) and that are needed to trigger IgE/FcεRI-mediated allergic reactions would never reach the levels needed to form immune complexes.

The observation that IgE-deficient mice can raise anaphylactic reactions should be viewed in the light of these species differences⁶⁵. In fact, this observation is unsurprising, as IgG immune complexes formed when challenging these mice intravenously with high antigen doses will cause FcγR-mediated cellular activation. Moreover, in a recent mouse study, a mechanism was proposed where binding of IgE/antigen to the inhibitory low-affinity IgG receptor FcγRIIb modulates the anaphylactic response⁶⁶. However, because IgE does not bind to FcγR in humans, the relevance of this observation to human allergy is limited.

This is not to argue that mouse models cannot be useful. It is important to be mindful of the fundamental differences between a mouse experiment and a human allergic response. First, IgE-mediated antigen presentation occurs in humans but not in rodents, and so it is unlikely that *in vivo* immunization of rodents (by injection or aerosol) will mimic the response seen in atopic humans. Second, the human allergic response results from monomeric IgE/FcεRI interactions and is not an immune complex disease. Most rodent experiments use amounts of antigen that will undoubtedly form IgG and IgE immune complexes, precisely because smaller amounts of antigen do not produce a biological response. Finally, IgE immune complexes bind to activating and inhibitory FcγR on rodent but not human mast cells. Thus there is a clear demarcation between IgE–FcεRI and IgG–FcγR networks in humans but not in rodents.

Interfering with FcεRI function

Modulation of FcεRI responses has long been considered as a therapeutic strategy in allergy. The field is now in a better position than ever to make rational decisions about drug design. There is a wealth of new information derived from the α-chain crystal structure that may be used to design peptide blockers of the IgE/FcεRIα chain interaction, or possibly of the proposed interaction between the D1/D2 cleft and the extracellular regions of the β- and γ-chains. Intracellular targets within signalling pathways are still not obvious. There are such significant parallels between the signalling cassettes used by FcεRI and other antigen receptors that specificity may be an insurmountable problem, although in humans the FcεRIβ/Lyn interaction occurs only in mast cells and basophils and may therefore offer a viable target. Finally, the recognition that the FcεRI expression levels correlate with serum IgE levels has led to an exciting new development. An anti-IgE immunotherapy is currently in phase-III human clinical trials, based on the concept that resulting decreased serum IgE will prevent levels of FcεRI on the surface of mast cells from accumulating, and therefore prevent a build-up of intensity in IgE-mediated cellular responses. □

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Therapeutic strategies for allergic diseases

Peter J. Barnes

Many drugs are now in development for the treatment of atopic diseases, including asthma, allergic rhinitis and atopic dermatitis. These treatments are based on improvements in existing therapies or on a better understanding of the cellular and molecular mechanisms involved in atopic diseases. Although most attention has been focused on asthma, treatments that inhibit the atopic disease process would have application to all atopic diseases, as they often coincide. Most of the many new therapies in development are aimed at inhibiting components of the allergic inflammatory response, but in the future there are real possibilities for the development of preventative and even curative treatments.

*Atopic diseases** account for a large proportion of health care spending in industrialized countries, as these conditions are common, persistent and currently incurable. There has been an enormous investment by the pharmaceutical companies in the search for new drugs in this profitable market. Current therapies for *asthma* and *rhinitis* are effective in most patients, whereas treatment for atopic dermatitis is less effective (Table 1). Anaphylactic shock is treatable with adrenaline (epinephrine) but there is a search for preventive therapies for susceptible patients. There is a need for new treatments to deal with more severe asthma that is currently not well controlled by high doses of inhaled corticosteroids and a need for a safe oral medication that would be effective in all atopic diseases, as they often occur together. The development of safe and effective oral therapies may depend on the discovery of treatments that are more specific for the atopic disease process, in order to avoid side effects.

New therapies for *allergic diseases* have developed by improving existing classes of drug or by discovering new classes of drug through research. Advances in understanding the molecular mechanisms of atopy have identified several new targets that might lead to new therapies for allergic diseases in the future¹.

This review summarizes some of the areas where new drugs are in development for atopic diseases. Most attention is focused on asthma, the atopic disease with the greatest clinical and economic impact, but treatments that are effective against the underlying atopic disease process might be effective against all atopic diseases, which commonly occur together.

Bronchodilators are used for symptom relief in asthma, but have no effect on the underlying inflammatory process. Inhaled β_2 -adrenergic agonists are safe and highly effective bronchodilators and it has proved impossible to find other classes of drug that are better. They relax airway smooth muscle by increasing the concentration of cyclic AMP and by opening potassium channels. Other classes of drug that mimic these effects, such as phosphodiesterase inhibitors or potassium channel openers, have not proved to be effective as bronchodilators, as side effects have limited the dose that can be administered¹. It is unlikely that novel bronchodilators that are more effective than β_2 -agonists will be developed and all of the attention has switched to the development of treatments that suppress or prevent the atopic inflammatory process.

I begin by discussing corticosteroids, which remain by far the most effective treatment for allergic diseases, as they provide the standard against which new treatments are judged. Many new classes of drug are in development and this very diversity highlights the fact that there are many possible therapeutic targets, most of which are suppressed by corticosteroids. The simplest approach is to

develop inhibitors of specific inflammatory *mediators* and, because many mediators have been implicated in atopic diseases, there are several such drugs in development. *Cytokines* have a critical role in the allergic inflammatory process and I review drugs that inhibit the cytokines that promote allergic inflammation, as well as cytokines that modulate the inflammatory process. I consider anti-inflammatory drugs with a broader spectrum of action, including broad-spectrum immunosuppressants, followed by drugs that may have a more selective inhibitory effect on the allergic process. Finally, I discuss treatment strategies that may prevent the development of allergic diseases.

Corticosteroids

Corticosteroids are the most effective treatment currently available for atopic diseases and high doses of oral corticosteroids would control almost every atopic patient. However, systemic side effects limit the dose that can be given over long periods, and this led to the development of topical steroids. There is little doubt that inhaled corticosteroids have revolutionized the treatment of asthma and are now first-line treatment for chronic asthma in patients of all ages and severity of disease². Nasal steroids are also the most effective treatment for allergic rhinitis³. Topical steroids for atopic dermatitis are associated with dermal atrophy, but and this has restricted the use of corticosteroids in the management of *eczema*. New-generation inhaled corticosteroids for asthma, including budesonide, fluticasone propionate and mometasone furoate, have a high level of anti-inflammatory action with minimal side effects, as the swallowed fraction of drug is largely removed by hepatic metabolism². However, these drugs are absorbed from the lung or nasal mucosa and so may have some systemic effects at high doses. There has therefore been a search for corticosteroids that are metabolized locally, so that there is less risk of systemic absorption from the respiratory tract or the skin. But these 'soft steroids', such as butixocort 21-propionate and tipredane, proved to have poor efficacy in clinical studies, as they are metabolized too rapidly before they exert their anti-inflammatory action. New soft steroids, such as ciclesonide, seem to be more promising⁴, and corticosteroids that are inactivated in plasma are now in development. Corticosteroids are highly effective in controlling atopic diseases, and there is increasing evidence that if started early they may prevent some of the irreversible airway narrowing in asthma². However, they do not cure the disease and allergic inflammation recurs when treatment is stopped.

Advances in understanding how corticosteroids suppress inflammation at a molecular level may lead to the development of safer steroids, or drugs that mimic their key anti-inflammatory actions. Corticosteroids bind to a cytosolic glucocorticoid receptor which translocates to the nucleus and binds as a homodimer to DNA to activate genes. The principal action of corticosteroids is to

* Terms in *italic* are defined in the glossary on p. B39.

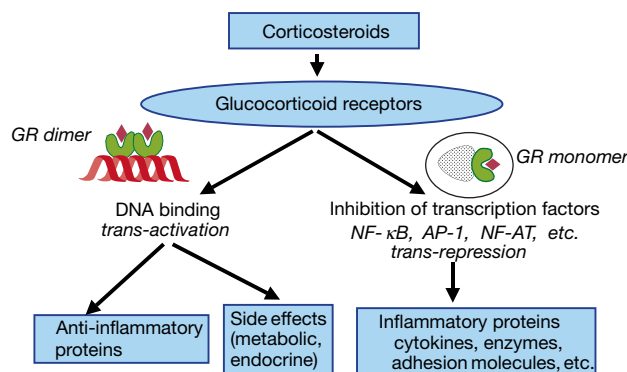


Figure 1 Dissociation of anti-inflammatory effects from side effects of corticosteroids. Corticosteroids bind to glucocorticoid receptors (GR) which dimerize to bind to DNA and increase transcription (*trans*-activation) and this mediates the systemic side effects of corticosteroids. Most of the anti-inflammatory effects of corticosteroids are mediated by inhibition of expression of inflammatory genes that are regulated by transcription factors,

such as activator protein-1 (AP-1), nuclear factor- κ B (NF- κ B) and nuclear factor of activated T cells (NF-AT), by interaction of GR monomers with these transcription factors (*trans*-repression). Some corticosteroids ('dissociated steroids') are able to selectively inhibit *trans*-repression to a greater extent than *trans*-activation.

suppress multiple inflammatory genes, including cytokines, inflammatory enzymes, adhesion molecules and inflammatory mediator receptors, and this is why corticosteroids are so effective in complex inflammatory conditions. Most of the anti-inflammatory actions of corticosteroids can be accounted for by inhibiting transcription factors, such as activator protein-1 (AP-1), nuclear factor- κ B (NF- κ B) and nuclear factor of activated T cells (NF-AT), that regulate inflammatory gene expression⁵. These effects are mediated, at least in part, by inhibition of core-histone acetylation **New corticosteroids.** Systemic side effects of corticosteroids are mediated largely through DNA binding, so that it may be possible to dissociate the anti-inflammatory effects, mainly mediated by transcription-factor inhibition, from their side effects (Fig. 1). Several dissociated corticosteroids have now been synthesized and a separation between *trans*-activation (DNA binding) and *trans*-repression (transcription-factor inhibition) has been demonstrated in gene reporter systems and in intact cells *in vitro*⁶. Whether this will translate to *in vivo* differences has not yet been determined and because all corticosteroids have to bind to a single class of glucocorticoid receptor, a large separation of effects may not be possible. Identification of the major targets for corticosteroid action, such as NF- κ B, activation of cAMP-response element binding protein (CREB)-binding protein (CBP) or acetylation of core histones, may be a more promising approach in the future.

Mediator antagonists

Many inflammatory mediators are involved in atopic diseases, and in asthma over 50 different mediators have been identified⁷. This implies that inhibitors of single mediators would be unlikely to be of major clinical benefit. Yet some mediator antagonists have been found to be useful in treating atopic diseases, indicating that these may have a more dominant role.

Antihistamines. Histamine H₁-receptor antagonists have a long history in the treatment of atopic diseases. Older antihistamines such as promethazine and chlorpheniramine, which caused sedation, have now been replaced by a new generation of antihistamines such as loratadine and fexafenadine, which are much less likely to sedate. These drugs are effective in rhinitis and reduce itch in atopic dermatitis, but have no clear benefit in asthma⁸. New antihistamines, such as cetirizine, ebastine and astemizole, have been claimed to have additional anti-asthma effects that are not mediated through H₁-receptor blockade. These effects include an inhibitory effect on eosinophil chemotaxis and adherence to endothelial cells, and inhibition of eosinophil recruitment into asthmatic airways after allergen challenge.

Antileukotrienes. Cysteinyl leukotrienes, generated from the rate-

limiting enzyme 5'-lipoxygenase (5-LO), are potent bronchoconstrictors and inducers of plasma exudation, and there is some evidence that they may promote eosinophilic inflammation⁹. 5-LO inhibitors (zileuton) and cysteinyl-leukotriene receptor (Cys-LT₁) antagonists (pranlukast, zafirlukast and montelukast) have been developed for the treatment of asthma, and possibly other atopic diseases. In challenge studies they reduce allergen- and exercise-induced asthma, as well as several other challenges. In clinical trials they improve asthma symptoms, lung function and reduce the need for rescue bronchodilator treatment⁹. A major advantage is that they are effective orally and, so far, there are no serious class-specific effects, although headache occurs in some patients. However, they are only weakly effective in asthma, although some patients (even with severe disease) may have a striking improvement. It is not yet possible to predict which patients will respond best, although there is some evidence that patients with aspirin-sensitive asthma do well, as may be expected from studies showing increased leukotriene production in these patients¹⁰. It is possible that genetic polymorphisms in the 5-LO pathway or Cys-LT₁ receptors might predict responders in the future¹¹. Although there are some reasons for thinking that antileukotrienes may have efficacy in allergic rhinitis, recent clinical studies have shown no benefit compared with nasal corticosteroids¹².

Other mediator inhibitors. Several other inhibitors of inflammatory mediators have been tested in asthma and rhinitis with disappointing effects, including inhibitors and receptor antagonists of thromboxane synthesis, antagonists of platelet activating factor, and bradykinin and tachykinin antagonists. Inhibitors of other mediators relevant to atopic diseases are in development. There is increased production of nitric oxide (NO) in asthma and rhinitis, with evidence for increased expression of inducible NO synthase (iNOS). NO may contribute to vasodilatation and plasma exudation and has been implicated in eosinophil recruitment and survival¹³. Selective inhibitors of iNOS are now in development

Table 1 Current therapies for atopic diseases

	Asthma	Allergic rhinitis	Eczema	Atopic conjunctivitis	Anaphylaxis
Topical corticosteroids	+++	+++	++	+++	—
Bronchodilators	+++	—	—	—	+++ (adrenaline)
Theophylline	++	—	—	—	—
Cromones	+	+	—	++	—
Antihistamines	—	++	+	—	+
Antileukotrienes	+	—	—	—	—

Key: +++, highly effective; ++, effective; +, weakly effective; — ineffective.

but have not yet reached clinical studies. Endothelins have been implicated in bronchoconstriction, smooth muscle hyperplasia and fibrosis, with effects mediated by endothelin-A and endothelin-B receptors¹⁴. Mixed endothelin antagonists have now been developed although they have not yet been tested in asthma; but if their principal effect is to inhibit airway remodelling they may prove difficult to test in clinical studies.

Tryptase inhibitors. Mast-cell tryptase has several effects on airways, including increasing responsiveness of airway smooth muscle to constrictors, increasing plasma exudation, potentiating eosinophil recruitment and stimulating fibroblast proliferation. Some of these effects are mediated by activation of the proteinase-activated receptor, PAR2. A tryptase inhibitor APC366 is effective in a sheep model of allergen-induced asthma¹⁵, but was only poorly effective in asthmatic patients¹⁶. More potent tryptase inhibitors and PAR2 antagonists are now in development.

Cytokine modulators

Multiple cytokines have been implicated in the pathophysiology of atopic diseases, although some cytokines have a more critical role in atopic inflammation¹⁷. There are several possible approaches to inhibiting specific cytokines. These include the use of drugs that inhibit cytokine synthesis (glucocorticoids, cyclosporin A and tacrolimus), humanized blocking antibodies to cytokines or their receptors, soluble receptors that 'mop up' secreted cytokines to receptor antagonists and drugs that block the signal-transduction pathways activated by cytokines (Fig. 2). On the other hand, there are cytokines that suppress the allergic inflammatory process and these may have therapeutic potential.

Anti-IL-5. Interleukin (IL)-5 is crucial in orchestrating the eosinophilic inflammation of asthma¹⁸ (Fig. 3). Blocking antibodies to IL-5 inhibit eosinophilic inflammation and *airway hyperresponsiveness* (AHR) in animal models of asthma, including primates. This blocking effect may last for up to 3 months after a single injection, making treatment of chronic asthma with such a therapy a feasible proposition. Humanized monoclonal antibodies to IL-5 have now been developed and a single injection reduces blood eosinophils for several weeks and prevents eosinophil recruitment into the airways after allergen challenge¹⁹. However, this treatment has no effect on the early or late response to allergen challenge or on AHR, indicating that eosinophils may not be critical for these responses. Similar findings have been reported previously in mice where anti-IL-5 antibodies reduced eosinophil responses to allergen, but not AHR, whereas AHR is reduced by anti-CD4 antibody, which depletes helper T cells (*Th cells*)²⁰. Long-term clinical studies are now in progress, particularly in patients with more severe disease. Non-peptidic IL-5-receptor antagonists would be an attractive alternative and there is a search for such compounds using molecular modelling of the IL-5-receptor α -chain and through large-scale throughput screening.

Anti-IL-4. IL-4 is critical for the synthesis of *immunoglobulin E* (IgE) by B lymphocytes and is also involved in eosinophil recruitment to the airways (see review by Corry & Kheradmand, this supplement). IL-4-receptor blocking antibodies inhibit allergen-induced AHR, *goblet-cell* metaplasia and pulmonary eosinophilia in a murine model²¹. Inhibition of IL-4 may therefore be effective in inhibiting allergic diseases, and soluble IL-4 receptors are in clinical development as a strategy to inhibit IL-4, with some preliminary evidence of efficacy by nebulization in moderate asthma²². IL-4 and the closely related cytokine IL-13 signal through a shared surface receptor that activates a specific transcription factor, signal transducer and activator of transcription 6 (Stat-6); deletion of the Stat-6 gene has a similar effect to knock-out of the IL-4 gene²³. This has led to a search for inhibitors of Stat-6, although it will be difficult to deliver these intracellularly. An endogenous inhibitor of STATs, suppressor of cytokine signalling (SOCS-1), is a potent inhibitor of IL-4 signalling pathways and offers a new therapeutic target²⁴.

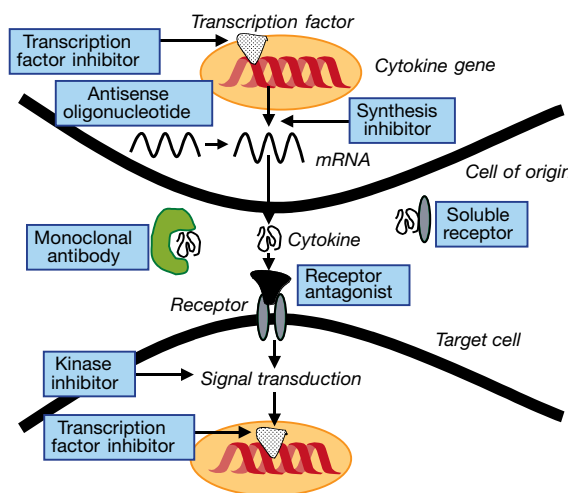


Figure 2 Strategies used to inhibit the production or effects of cytokines.

Anti-IL-13. There is increasing evidence that IL-13 in mice mimics many of the features of asthma, including AHR and mucus hypersecretion, independently of eosinophilic inflammation²⁵. It is also a potent inducer of eotaxin secretion from airway epithelial cells²⁶. IL-13 signals through the IL-4-receptor α -chain, but may also activate different intracellular pathways²⁷, so that it may be an important target for the development of new therapies.

Anti-TNF. Tumour-necrosis factor (TNF)- α is expressed in asthmatic airways and may be important in amplifying asthmatic inflammation, through the activation of NF- κ B, AP-1 and other transcription factors. In rheumatoid arthritis and inflammatory bowel disease a blocking antibody to TNF- α (infliximab) has produced remarkable clinical responses, even in patients who are relatively unresponsive to steroids²⁸. Such antibodies or soluble TNF receptors are a logical approach to asthma therapy, particularly in patients with severe disease. There is also a search for small-molecule inhibitors of TNF, of which the most promising are inhibitors of TNF- α converting enzyme as these could be given orally.

Chemokine inhibitors. Chemokines are chemoattractant cytokine molecules. Chemokines such as RANTES, MCP-3, MCP-4 and eotaxin may be crucial in the recruitment of eosinophils in atopic patients; all of these chemokines act on a common receptor, the CCR3 receptor, that is expressed predominantly on eosinophils²⁹. An antibody to human CCR3 blocks the chemotactic response of human eosinophils to all chemokines³⁰. The modified chemokine met-RANTES similarly blocks CCR3 receptors and inhibits eosinophil chemotactic responses to chemokines³¹. Chemokine receptors are G-protein-coupled receptors with the typical seven-transmembrane-spanning segments and are therefore have a simpler structure than the receptors for other cytokines. Non-peptide inhibitors of CCR3 receptors are now in development and should be relatively safe as the distribution of CCR3 receptors is restricted to eosinophils, Th2 cells and *basophils*.

Anti-inflammatory cytokines. Some cytokines have anti-inflammatory effects in atopic inflammation and therefore have therapeutic potential³². Although it may not be feasible or cost-effective to administer these proteins as long-term therapy, it may be possible to develop drugs that increase the release of these endogenous cytokines or activate their receptors and specific signal-transduction pathways. IL-1-receptor antagonist (IL-1ra) binds to IL-1 receptors and blocks the action of IL-1 β . In experimental animals it reduces AHR³³ and clinical studies are underway.

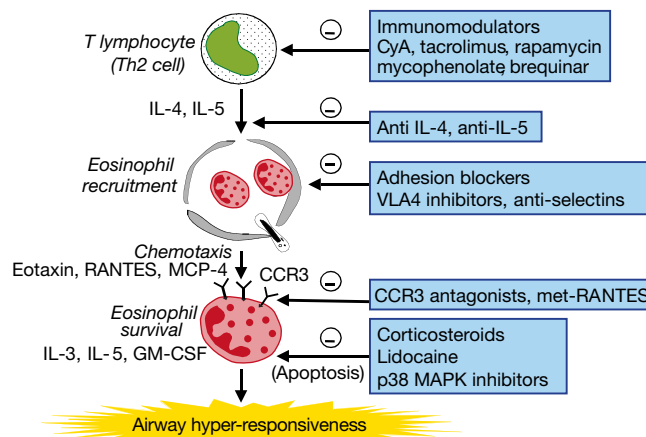


Figure 3 Inhibition of eosinophilic inflammation. Several strategies are possible to inhibit eosinophilic inflammation in tissues, including immunomodulators, inhibitors of driving cytokines (IL-4 and IL-5), inhibition of critical adhesion molecules (VLA4, selectins and

ICAM-1), blockade of chemokine receptors on eosinophils (CCR3) and induction of apoptosis.

IL-10 is a potent anti-inflammatory cytokine that inhibits the synthesis of many inflammatory proteins, including cytokines (TNF- α , granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-5 and chemokines) and inflammatory enzymes (iNOS) that are overexpressed in asthma³⁴. Indeed, there may be a defect in IL-10 transcription and secretion from macrophages in asthma^{35,36}. In sensitized animals, IL-10 is effective in suppressing the inflammatory response to allergen, indicating that IL-10 might be defective in atopic diseases. Recombinant human IL-10 has proved to be effective in controlling inflammatory bowel disease, where similar cytokines are expressed, and may be given as a weekly injection³⁷. In the future, drugs that activate the unique signal-transduction pathways activated by the IL-10 receptor or drugs that increase endogenous production of IL-10 may be developed. In mice, drugs that elevate cAMP increase IL-10 production, but this does not seem to be the case in human cells³⁸.

Interferon- γ (IFN- γ) inhibits Th2 cells and should therefore reduce atopic inflammation. In sensitized animals, nebulized IFN- γ inhibits eosinophilic inflammation induced by allergen exposure³⁹ (see review by Corry & Kheradmand, this supplement). But administration of IFN- γ by nebulization to asthmatic patients did not significantly reduce eosinophilic inflammation, although this may be due to the difficulty in obtaining a high enough concentration locally in the airways⁴⁰. Allergen immunotherapy increases IFN- γ production by circulating T cells in patients with clinical benefit⁴¹ and it increased the number of cells expressing IFN- γ in nasal biopsies of patients with allergic rhinitis⁴².

IL-12 is the endogenous regulator of Th1-cell development and determines the balance between Th1 and Th2 cells⁴³. IL-12 administration to rats inhibits allergen-induced inflammation⁴⁴ and inhibits sensitization to allergens. IL-12 releases IFN- γ , but has additional effects on T-cell differentiation. Recombinant human IL-12 has been administered to humans and has several toxic effects which are diminished by slow escalation of the dose. In mice, administration of an IL-12-allergen fusion protein results in the development of a specific Th1 response to allergens rather than the normal Th2 response with IgE formation⁴⁵. This indicates the possibility of using IL-12 to provide a more specific immunotherapy, which might even be curative if applied early in the course of the atopic disease.

New anti-inflammatory drugs

There has been an intensive search for anti-inflammatory treatments that are as effective as glucocorticoids but with fewer side effects. Whereas one approach is to seek corticosteroids with a

greater therapeutic effect, other approaches involve developing different classes of anti-inflammatory drugs.

Phosphodiesterase-4 inhibitors. Phosphodiesterases (PDEs) break down cyclic nucleotides that inhibit cell activation and at least nine families of enzymes have now been discovered. Theophylline, long used as an asthma treatment, is a weak but non-selective PDE inhibitor. PDE4 is the predominant family of PDEs in inflammatory cells, including mast cells, eosinophils, T lymphocytes, macrophages, and structural cells such as sensory nerves and epithelial cells⁴⁶. This has suggested that PDE4 inhibitors would be useful as an anti-inflammatory treatment in atopic disease, particularly as there is some evidence for over-expression of PDE4 in cells of atopic patients⁴⁷. In animal models of asthma, PDE4 inhibitors reduce eosinophil infiltration and AHR responses to allergen⁴⁶. Several PDE4 inhibitors have been tested in asthma, but with disappointing results. One PDE4 inhibitor, CDP840, had a marginal inhibitory effect on the late response to allergen, but is not being further developed⁴⁸. However, most of the PDE4 inhibitors so far tested clinically have had unacceptable side effects, particularly nausea and vomiting, and such side effects have limited the use of theophylline.

Several steps may be possible to overcome these problems. It is possible that vomiting is due to inhibition of a particular subtype of PDE4. At least four human PDE4 genes have been identified and each has several splice variants^{46,49}. This raises the possibility that subtype-selective inhibitors may be developed that may preserve the anti-inflammatory effect, while having less propensity to side effects. PDE4D seems to be of particular importance in inflammatory cells, such as T lymphocytes and eosinophils, and may be a more specific target⁵⁰; subtype-selective PDE4 inhibitors are now in development.

Transcription-factor inhibitors. Transcription factors, such as NF- κ B and AP-1, are important in the orchestration of asthmatic inflammation^{51,52} and this has prompted a search for specific blockers of these transcription factors. NF- κ B is inhibited naturally by the inhibitory protein I κ B, which is degraded after activation by specific kinases. Inhibitors of I κ B kinases or the proteasome, the multifunctional enzyme that degrades I κ B, would thus inhibit NF- κ B and there is a search for such inhibitors. There are some naturally occurring inhibitors of NF- κ B, such as the fungal product gliotoxin, although this compound is toxic. There are concerns that inhibition of NF- κ B may cause side effects such as increased susceptibility to infections, which as been observed in gene-disruption studies when components of NF- κ B are inhibited⁵¹.

Cyclosporin A and tacrolimus inhibit T-lymphocyte function by blocking the transcription factor NF-AT by blocking activation of

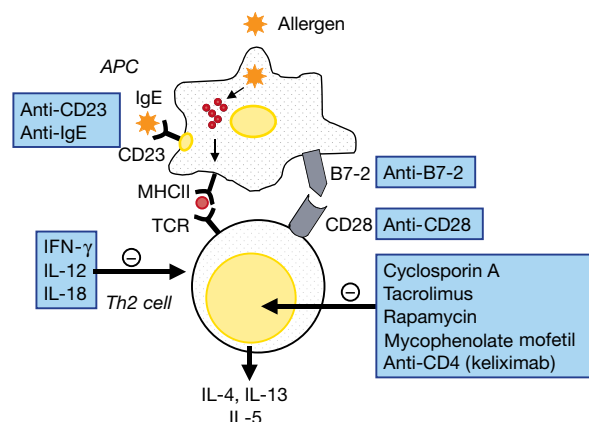


Figure 4 Inhibition of antigen-presenting cells (APCs) and Th2 lymphocytes. Therapies are based on inhibition of co-stimulatory molecules (B7-2 and CD28), inhibition of

IgE-driven APCs, and the use of non-selective immunomodulators or cytokines that tip the balance away from Th1 cells towards Th2 cells

calcineurin. This results in suppression of IL-2, IL-4, IL-5 and GM-CSF and so offers therapeutic potential in atopic diseases (see below).

MAP kinase inhibitors. There are three major mitogen-activated protein (MAP) kinase pathways and there is increasing recognition that these pathways are involved in chronic inflammation⁵³. There has been particular interest in the p38 MAP kinase pathway that is blocked by a new class of drugs, the cytokine suppressant anti-inflammatory drugs (CSAIDs), such as SB203580 and RWJ67657. These drugs inhibit the synthesis of many inflammatory cytokines, chemokines and inflammatory enzymes. They appear to have a preferential inhibitory effect on synthesis of Th2 compared with Th1 cytokines, indicating their potential application in the treatment of atopic diseases⁵⁴. Furthermore, p38 MAP kinase inhibitors have also been shown to decrease eosinophil survival by activating apoptotic pathways⁵⁵. Whether this new class of anti-inflammatory drugs will be safe in long-term studies remains to be established.

Tyrosine kinase inhibitors. Syk kinase is a protein tyrosine kinase that has a pivotal role in signalling of the high-affinity IgE receptor (FcεRI) in mast cells. In *syk*-deficient mice, mast-cell degranulation is inhibited, indicating that this might be an important potential target for the development of mast-cell stabilizing drugs⁵⁶. Syk is also involved in antigen-receptor signalling of B and T lymphocytes and in eosinophil survival in response to IL-5 and GM-CSF⁵⁷, so that *syk* inhibitors might have several useful beneficial effects in atopic diseases. Another tyrosine kinase *lyn* is upstream of *syk*, and an inhibitor of *lyn* kinase, PP1, has an inhibitory effect on inflammatory and mast-cell activation⁵⁸. But as *lyn* and *syk* are widely distributed in the immune system, there are doubts about the long-term safety of selective inhibitors.

Immunosuppressants. T lymphocytes may be important in initiating and maintaining the inflammatory process in atopy through the release of cytokines that result in eosinophilic inflammation, indicating that T-cell inhibitors may be useful in controlling asthmatic inflammation. The non-specific immunomodulator cyclosporin A reduces the dose of oral steroids needed to control asthma in patients with severe asthma⁵⁹, but its efficacy is very limited⁶⁰, and side effects, particularly nephrotoxicity, limit its clinical use. The possibility of using inhaled cyclosporin A is now being explored, as in animal studies the inhaled drug is effective in inhibiting the inflammatory response in experimental asthma⁶¹. Immunomodulators, such as tacrolimus (FK506) and rapamycin, seem to be more potent but are also toxic and may offer no real advantage. Topical tacrolimus seems to be effective in atopic dermatitis and is well tolerated⁶². Novel immunomodulators that inhibit purine or pyrimidine pathways, such as mycophenolate mofetil, leflunomide and brequinar sodium, may be less toxic and therefore of greater potential value in asthma therapy⁶³.

One problem with these non-specific immunomodulators is that they inhibit both Th1 and Th2 cells, and therefore do not restore the imbalance between these cells in atopy. They also inhibit suppressor T cells (Tc1 cells) that may modulate the inflammatory response. Selective inhibition of Th2 cells may be more effective and better tolerated and there is now a search for such drugs.

Cell adhesion blockers. Infiltration of inflammatory cells into tissues is dependent on adhesion of blood-borne inflammatory cells to endothelial cells before migration to the inflammatory site⁶⁴. This depends upon specific glycoprotein adhesion molecules, including integrins and selectins, on both leukocytes and endothelial cells, which may be upregulated or show increased binding affinity in response to various inflammatory stimuli such as cytokines or lipid mediators. Monoclonal antibodies which inhibit these intracellular adhesion molecules (ICAMs) may prevent inflammatory cell infiltration. Thus a monoclonal antibody to ICAM-1 on endothelial cells prevents the eosinophil infiltration into airways and the increase in bronchial reactivity after allergen exposure in sensitized primates⁶⁵, although this has not been found in other species⁶⁶. The interaction between very late antigen (VLA)-4 and vascular cell-adhesion molecule (VCAM)-1 is important for eosinophil inflammation and humanized antibodies to VLA-4 (α4α1) have been developed⁶⁷ (Fig. 3). Small-molecule peptide inhibitors of VLA-4 have been developed which are effective in inhibiting allergen-induced responses in sensitized sheep⁶⁸. Inhibitors of selectins, particularly L-selectin, based on the structure of sialyl-Lewis^x, inhibit the influx of inflammatory cells in response to inhaled allergen in sensitized sheep⁶⁹ and inhibit adhesion of human eosinophils *in vitro*⁷⁰. Although blocking adhesion molecules is an attractive new approach to the treatment of inflammatory disease, there may be potential dangers in inhibiting immune responses leading to increased infections and increased risks of neoplasia.

Specific anti-allergic drugs

Although corticosteroids are effective in controlling atopic diseases, there are continuing concerns about systemic side effects when high doses are needed. This has prompted a search for more selective anti-inflammatory agents that would selectively target the atopic disease process.

Cromones. The cromones (sodium cromoglycate and nedocromil sodium) are the most specific anti-allergic drugs so far discovered. Topical application is effective in asthma, rhinitis and allergic conjunctivitis, but the effects are less marked than seen with topical steroids and they are effective only in mild disease. Cromones seem to have a specific action on allergic inflammation, yet their molecular mechanism of action remains obscure. Although it was believed that the primary mode of action of cromones involved inhibiting

mast-cell mediator release, it has now been shown that they affect several other inflammatory cells and sensory nerves, including certain types of chloride channels that are expressed in mast cells⁷¹. Sodium cromoglycate phosphorylates moesin, a specific cytoskeletal protein in mast cells, indicating a possible mechanism that may inhibit degranulation⁷². Both cromoglycate and nedocromil sodium must be given topically and all attempts to develop orally active drugs of this type have been unsuccessful, possibly suggesting that topical administration is critical to their efficacy.

The diuretic furosemide shares many of the actions of cromones in inhibiting indirect bronchoconstrictor challenges (such as allergen, exercise, cold air, adenosine and metabisulphite) but not direct bronchoconstriction (such as histamine and methacholine) when given by inhalation⁷³. The mechanism of action of furosemide is not shared by the more potent loop-diuretic bumetanide, indicating that some other mechanism than the inhibition of the $\text{Na}^+/\text{K}^+/\text{Cl}^-$ co-transporter must be involved. This probably involves inhibition of the same chloride channel that is inhibited by cromones. Furosemide itself does not seem to be particularly effective when given regularly by metered dose inhaler in asthma⁷⁴, but it is possible that more potent and long-lasting chloride-channel blockers might be developed in the future.

Co-stimulation inhibitors. Co-stimulatory molecules may be crucial in augmenting the interaction between antigen-presenting cells (APCs) and CD4^+ T lymphocytes (see review by Corry & Kheradmand, this supplement). The interaction between B7 and CD28 may determine whether a Th2-type cell response develops, and there is some evidence that B7-2 (CD86) skews towards a Th2 response (Fig. 4). Blocking antibodies to B7-2 inhibit the development of specific IgE, pulmonary eosinophilia and AHR in mice, whereas antibodies to B7-1 (CD80) are ineffective⁷⁵. A molecule on activated T cells, CTLA4, seems to act as an endogenous inhibitor of T-cell activation and CTLA4-Ig, a soluble fusion-protein construct, is also effective in blocking AHR in a murine model of asthma⁷⁶. Anti-CD28, anti-B7-2 and CTLA4-Ig also block the proliferative response of T cells to allergen⁷⁷, indicating that these are potential targets for new therapies that should be effective in all atopic diseases.

Th2-cell inhibitors. Non-selective T-cell suppressants, such as cyclosporin A and tacrolimus, may be relatively ineffective in asthma as they inhibit all types of T cell. CD4^+ T cells have been implicated in asthma and a chimaeric antibody directed against CD4^+ (keliximab), which reduces circulating CD4^+ cells, seems to have some beneficial effect in asthma⁷⁸, although long-term safety of such a treatment might be a problem. Furthermore, there is increasing evidence that CD8^+ cells (Tc2 cells), through release of IL-5 and other cytokines, might also be involved in atopic diseases, particularly in response to infections with certain viruses⁷⁹. There has been a search for selective inhibitors of Th2 cells by identifying features that differentiate Th1 and Th2 cells. The transcription factor GATA-3 seems to be of particular importance in murine and human Th2 cells^{80,81} and may be a target for selective immunomodulatory drugs. However, an argument against strategies to control atopic disease by targeting Th2 cells is that chronic stimulation (by exposure to allergen) results in cells that are relatively resistant to immune suppression⁸².

Anti-IgE. Because release of mediators from mast cells in asthma is IgE-dependent (see review by Turner & Kinet, this supplement), an attractive approach is to block the activation of IgE using blocking antibodies that do not result in cell activation. A humanized murine monoclonal antibody directed to the FcεRI-binding domain of human IgE (rhuMAB-E25) reduces allergen-specific IgE after intravenous administration⁸³. RhuMAB-E25 also reduces early and late responses to inhaled allergen and eosinophil counts in induced sputum⁸⁴. Although a reduction in early response to allergen, which is due to mast-cell activation through bound IgE, is expected, the reduction in the late response and in sputum eosinophils is

unexpected, but it may be explained by blocking the effect of IgE on low-affinity IgE receptors (CD23) on APCs. Anti-IgE in mice inhibits IL-4 and IL-5 secretion and pulmonary eosinophilia by blocking Th2-cell activation in response to allergen, and this is mimicked by an anti-CD23 antibody⁸⁵. Clinical studies with rhuMAB-E25 are now in progress⁸⁶. Although injections of antibody may not be feasible for the long-term treatment of mild asthma, this could be a realistic therapy for patients with more severe forms of asthma or atopic dermatitis, in whom high IgE levels may be found.

Preventive strategies

Atopy seems to be due to immune deviation from Th1 to Th2 cells, which may arise because of a failure to inhibit the normal Th2 preponderance at birth, which in turn may result from environmental factors such as the Th1 response to infectious agents (see review by Holt *et al.*, this supplement).

Specific allergen vaccination (immunotherapy). Subcutaneous injection of small amounts of purified allergen has been used for many years in the treatment of allergy. It is effective in the treatment of insect venom anaphylaxis and hay fever, and may induce prolonged remission⁸⁷, but is less effective in asthma. The molecular mechanism of desensitization is unknown. Cloning of several common allergen genes has made it possible to prepare recombinant allergens for injection, although this purity may detract from their allergenicity as most natural allergens contain several proteins. Intramuscular injection of rats with plasmid DNA expressing house dust mite allergen results in its long-term expression and prevents the development of IgE responses to inhaled allergen⁸⁸. This suggests that allergen gene immunization might be a useful therapeutic strategy in the future. The major allergens of peanuts, which are responsible for an increasing number of severe anaphylactic reactions, has been cloned and oral administration of microparticles of DNA complexed to chitosan in mice produces intestinal epithelial expression of the allergen and prevention of anaphylaxis after oral challenge⁸⁹.

Peptide immunotherapy. Small peptide fragments of allergen (epitopes) are able to block allergen-induced T-cell responses without inducing anaphylaxis⁹⁰. T-cell-derived peptides from cat allergen (fel d1) seem to be effective in blocking allergen responses to cat dander⁹¹, but may induce an isolated late response to allergen by direct T-cell activation⁹².

Vaccination. A relative lack of infections may be a factor that influences the development of atopy in genetically predisposed individuals. This leads to the concept that vaccination may induce protective Th1 responses to prevent sensitization and thus prevent the development of atopic diseases (see review by Holt *et al.*, this supplement). Bacille Calmette-Guérin (BCG) vaccination has been associated with a reduction in atopic diseases in Japan⁹³, but this has not been confirmed in a Swedish population⁹⁴. BCG inoculation in mice, delivered 14 days before allergen sensitization, reduced the formation of specific IgE in response to allergen and the eosinophilic response and AHR responses to allergen, with an increase in production of IFN-γ⁹⁵. This has prompted several clinical trials of BCG to prevent the development of atopy. Similar results have been obtained in mice with the a single injection of heat-killed *Mycobacterium vaccae*, another potent inducer of Th1 responses⁹⁶, and with *Listeria*. *Lactobacillus acidophilus* in yoghurt, another potential means of tipping back the balance from Th2 to Th1 cells, weakly increases IFN-γ formation in adult asthmatic patients⁹⁷. Immunostimulatory DNA sequences, such as unmethylated cytosine-guanosine dinucleotide-containing oligonucleotides (CpG ODN), are also potent inducers of Th1 cytokines and, in mice, administration of CpG ODN increases the ratio of Th1 to Th2 cells, decreases formation of specific IgE and reduces the eosinophilic response to allergen, an effect that lasts for over 6 weeks⁹⁸. These promising animal studies encourage the possibility that vaccination might prevent or cure atopic diseases in the future.

Gene therapy

Because atopic diseases are polygenic, it is unlikely that gene therapy will be of value in long-term therapy (see review by Cookson, this supplement). However, identifying the genes involved in atopic diseases and in disease severity may identify new molecular targets and may also predict the response to different forms of therapy (pharmacogenetics). Transfer of anti-inflammatory genes may provide specific anti-inflammatory or inhibitory proteins in a convenient manner and gene transfer has been shown to be feasible in animals using viral vectors⁹⁹. Anti-inflammatory proteins relevant to asthma include IL-10, IL-12 and I κ B. Antisense oligonucleotides may switch off specific genes, but there are considerable problems in getting these molecules into cells. An inhaled antisense oligonucleotide directed against the adenosine A₁-receptor has been shown to reduce AHR in a rabbit model of asthma, demonstrating the potential of this approach in treating asthma¹⁰⁰. Suitable target genes may be IL-4 or IL-5. Considering the practical problems encountered by gene therapy, this approach is unlikely in the foreseeable future, other than for proof-of-concept studies.

Conclusions

Many different therapeutic approaches to the treatment of atopic diseases may be possible, yet there have been few new drugs that have reached the clinic. Topical glucocorticoids are particularly effective as chronic treatment in atopic diseases and suppress the underlying inflammatory process. Advances in therapy would be facilitated through the development of more specific anti-allergic drugs that lack side effects. If these treatments can be taken orally this would treat asthma, rhinitis and eczema, which often coincide. The possibility of developing a 'cure' for atopy is remote, but strategies to inhibit the development of sensitization in early childhood offer such a prospect in the future. □

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Allergy and asthma: Glossary

The following glossary provides definitions of terms used frequently throughout this collection of reviews. Within each review, glossary entries are set in *italic font* at first mention.

Adjuvant. Any agent that augments or facilitates a desired immune outcome. Immune adjuvants amplify immune responses to discrete antigens and often determine the direction (Th1 or Th2) of the response.

Airway or bronchial hyperresponsiveness.

Characteristic physiological abnormality in asthma with exaggerated airway narrowing in response to bronchoconstrictor stimuli.

Allele. Different coding sequences for the same gene occurring in the same species.

Allergen. A subset of antigens that strongly elicits Th2 cells, IgE and thus atopic disease. Common allergen sources include house dust mite, grass pollens and animal danders (sheddings from skin and fur).

Allergy/Allergic disease. Originally intended to mean 'deviation from the original state' when vaccination and injection with proteins and sera were seen to lead to a variety of harmful immune reactions. Now used to describe Th2-associated immune reactions to common environmental proteins, known as allergens. (In present usage, allergy is virtually synonymous with atopy.)

Anaphylaxis. A hypersensitivity response marked by systemic signs and symptoms (for example, shortness of breath and lowered blood pressure), often accompanied by urticaria.

Anergy. Suppressed functional state.

Antigen. Any substance that elicits an immune reaction involving B or T cells.

Antigenic determinant. A part of a foreign protein that is recognized by the host's immune system.

Asthma. An inflammatory disease of the airways of the lung, characterized by intermittent airway narrowing and variable symptoms of chest tightness, wheeze and shortness of breath.

Atopic asthma. Asthma with a strong allergic component. Most (95%) of childhood asthma is atopic, but the proportion is far more variable in adults with asthma.

Atopy/Atopic disease. A word meaning 'strange disease' coined to describe the familial

syndrome of asthma and hay fever. Eczema is part of the same syndrome. The atopic state can be recognized by elevated serum IgE levels, and positive skin or ELISA tests which detect IgE directed against allergens. (Now virtually synonymous with allergy.)

Basophil. Granulocytic leukocyte, related to mast cells, and involved in IgE-mediated allergic responses.

Chemokine. Mediator that attracts inflammatory cells to a site of secretion.

Cytokine. A small secreted protein that mediates many functions of the immune system.

Dendritic cell. Antigen-presenting cell, related to but distinct from macrophages.

Eczema. An itchy red rash with an easily broken surface that has a predisposition for flexures in the skin.

Eosinophil. Granulocytic leukocyte having a key role in late phase of allergic response, particularly through interleukin (IL)-5-mediated activation and subsequent mediator secretion.

Epitope. A single antigenic determinant. T and B cells may react to different epitopes.

Genetic heterogeneity. A condition in which a disease state is caused by variation in more than one gene.

Goblet cell. Mucus-secreting cell in the airway epithelium.

Hypersensitivity. Abnormal or excessive responsiveness to a sensitizing agent, usually requiring previous exposure.

Immune complex. Large complexes of antigen and antibody that can lead to a disease state.

Immunoglobulin E (IgE). A major class of immunoglobulin. IgE is the allergic antibody that binds to receptors on the surface of mast cells. The presence of allergen in a sensitized individual causes crosslinking of the IgE/receptor complex and the release of pro-inflammatory factors from mast-cell granules.

Langerhans cell. Antigen-presenting cell located in the epidermis.

Linkage disequilibrium. The chromosomal association of distinctive alleles of individual polymorphisms with alleles of closely neighbouring polymorphisms in DNA.

Macrophage. Phagocytic leukocytes responsible for antigen presentation to T cells.

Mast cell. Granulocytic leukocyte largely responsible for acute allergic reactions particularly through IgE-mediated histamine secretion.

Mediator. Secreted molecule with biological activity.

Monocyte. Immature macrophage.

Natural killer cell. An immune cell capable of recognizing and destroying certain cancerous and virally infected cells without requiring previous sensitization.

Neutrophil. Granulocytic leukocyte that contains a distinct set of mediators involved in the inflammatory response.

Oedema. Inflammation-induced swelling.

Pleiotropy. The condition whereby a single genetic variant can cause a wide variety of symptoms and signs of disease.

Polymorphism. Literally 'many forms'. Used to describe variations in DNA, both within and outside genes.

Rhinitis. Atopic inflammation in the nose. Seasonal rhinitis is known as hay fever.

Th1/Th2 cell (helper T cell 1/helper T cell 2). Terminally differentiated CD4⁺ T cells characterized by distinct secreted products and immune functions. Th1 cells secrete cytokines that predominantly activate intracellular antimicrobial defences; Th2 cells secrete cytokines that activate extracellular defence mechanisms and are associated with atopic disease.

Tolerance. Immunological unresponsiveness; a state in which an antigen, recognized under some conditions, is ignored by the immune system.

Urticaria (hives). A hypersensitivity response of the skin marked by raised, swollen, erythematous patches and intense itching.

Schering-Plough and allergic dis-

Allergic disease is common, and although long considered trivial, recent research suggests that it disrupts many everyday functions in allergy sufferers and can have a significant effect on their quality of life. Furthermore, loss of productivity in the workplace and educational impairment at school, as a result of allergic symptoms, has a significant economic cost. Anti-allergy drugs are an important element in the management of allergic diseases and historically there has been a clear evolution in the approach to the discovery of new drugs.

Histamine (H₁)-receptor antagonists and corticosteroids have been the mainstay of the drug treatment of allergy over the past 50 years and Schering-Plough scientists have made their own contributions to this area. Chlorpheniramine and polaramine, potent H₁-receptor antagonists were discovered in the 1940s, and prednisone and prednisolone, prototypic oral corticosteroids, in the 1950s. Advances in medicinal chemistry and pharmacology, coupled with a greater understanding of drug metabolism and pharmacokinetics, have led to the improvements in the anti-allergy medicines widely used today. For instance, once-daily dosing and lack of sedation are important characteristics of today's most widely used H₁-receptor antagonists. Similarly, the improved therapeutic index of intranasal corticosteroids is due to increased topical potency and low systemic bioavailability.

Mast cells and histamine release were the primary focus of drug discovery for many years. Over the past quarter century both academic institutions and the pharmaceutical industry have invested considerable

effort in understanding the genetics and pathophysiology underlying allergic diseases and as a result many potential targets for new drugs have been identified. For example, in 1986, Mosmann and Coffman (*J. Immunol.* 136, 2348–2357; 1986) from DNAX Institute of Molecular Biology, a subsidiary of Schering-Plough Research Institute, proposed that T helper cells could be divided into different types depending on their pattern of cytokine secretion. Their theory predicted that cytokines secreted by type-2 helper T cells were important in allergic inflammation. This insight provided not only a better understanding of the immune processes underlying allergy, but also a valuable framework for the identification of new drug targets by Schering-Plough and others.

Drug discovery and development is now in the midst of a major revolution driven by major advances in technology. The ever increasing amount of information on genes and their products, which resides in highly sophisticated databases, is generating many new genomic targets for high throughput robotic screening. Application of high throughput synthesis and structure-based drug design is rapidly providing novel chemical leads. Thus, the prospects for new therapeutics for allergic disease in the future are excellent.

Schering-Plough has a major commitment to the improved treatment of allergic diseases through cutting-edge basic research, efficient drug development and comprehensive educational and pharmacoeconomic programmes. Sponsorship of this valuable supplement is part of this mission.

Francis Cuss, Vice President, Discovery Research (Allergy/Immunology)



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